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United States
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Proceedings of the 1982 Sugar Processing Research Conference

aTP375.T4

April 29-May 1, 1982
Atlanta, Georgia

Proceedings of the 1982
Sugar Processing Research Conference

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Sponsored by

Sugar Processing Research, Inc.

and

Southern Regional Research Center
U.S. Department of Agriculture

Agricultural Research Service
U.S. Department of Agriculture
1983

The Proceedings of the Sugar Processing Research Conference (ISSN 0730-6490), of which this is the first issue, continues the Proceedings of the Technical Session on Cane Sugar Refining Research (ISSN 0197-7288), which was published every other year from 1964 to 1980. To place a standing order for this series, write the publisher. (The address is at the bottom of the page.) For individual copies of this volume as well as back issues of the old series, write the Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 19687, New Orleans, La. 70179.

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Proceedings of the 1982 Sugar Processing Research Conference,
April 29-May 1, 1982, Atlanta, Georgia. Issued March 1983.

Published by Agricultural Research Service (Southern Region),
U.S. Department of Agriculture, P.O. Box 53326, New Orleans, La.
70153.

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FOREWORD

This conference is sponsored jointly by Sugar Processing Research, Inc. and the Southern Regional Research Center of the U.S. Department of Agriculture. The program was arranged by Dr. Margaret A. Clarke, Dr. Frank G. Carpenter, and Mary An Godshall. The conference coordinator was Shirley T. Saucier.

This is one of a series of conferences held in alternate years to provide a forum for exchange of information among technical leaders of the sugar industry and to report on new and important developments.

Margaret A. Clarke
Managing Director
Sugar Processing Research, Inc.

Michael C. Bennett
President
Sugar Processing Research, Inc.

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U.S. Department of Agriculture

AN HPLC STUDY OF THE CHANGES IN COLORANT COMPOSITION FOLLOWING FACTORY DECOLORISATION OF RAW LIQUORS WITH BONE CHAR, RESIN AND GRANULAR CARBON

Nancy H Paton and Peter Smith

CSR Limited

INTRODUCTION

Research by a number of workers has shown that cane sugar colour is a complex mixture of components which are chemically diverse. With the application of new analytical techniques the chemical complexity of cane sugar colour is being progressively resolved. For example, gel filtration was used to separate cane sugar colour into fractions differing in molecular weight (N H Smith 1966). A more effective separation of colour components was later achieved by a combination of solvent extraction and high voltage electrophoresis (Farber and Carpenter 1971). However these methods were qualitative only.

More recently, near quantitative recovery of colorants from mill syrup was a major advance in the study of the chemistry of cane sugar colorants (Linecar et al. 1978). This method paved the way for the study of properties of single colour components and their quantification by analytical techniques such as high performance liquid chromatography (HPLC).

At the same time, the superior resolution of cane sugar colorants by HPLC compared with gel filtration was first demonstrated by C & H Sugar Company (N H Smith 1977). However, attempts to identify the components of the many peaks in HPLC profiles were unsuccessful.

In the interim, we have spent considerable time developing a suitable HPLC technique for the separation of colorants. Our HPLC method was developed primarily for the separation of flavonoids as we have found this class of colorant to be a major contributor to the colour of white sugar. Furthermore, we have been able to utilise our chromatographic knowledge of cane flavonoids (Williams et al. 1974) and phenolic colour

precursors (Paton 1977, Curtin and Paton 1980) to identify a number of the many peaks in HPLC colorant profiles of cane sugar products.

This paper examines the changes in colorant composition after decolorisation with three different processes. These were bone char, ion exchange resin and granular activated carbon. All three processes are now used in CSR refineries. In all cases the preceeding operations consist of affination, carbonatation and pressure filtration to produce raw liquor. The changes in colorant composition were monitored by HPLC on colour solids recovered from raw liquors, the decolorised liquors (fine liquors) and the corresponding granulated sugars. The results showed firstly the relative affinity of each of the decolorisers for a range of cane sugar colorants in raw liquor and secondly the effect of each type of decoloriser on the colorant composition of refined sugar. By using apigenin as a standard an approximate estimation was made of the concentration of flavonoid colorants in the process samples.

EXPERIMENTAL

Two main experimental procedures were employed in the colour study: the recovery of colorants from the refinery samples and the resolution of the components of the "recovered" colorants by HPLC.

Colour Solids Recovery

The preparation of colour solids involved the adsorption of colorants from a sugar solution on a column of the resin XAD-2, washing off the sugar, and desorption of the colorants. Details of the method were reported earlier. (Linecar et al. 1978). Two minor modifications to the procedure were:

- o the use of smaller Pharmacia C16/20 columns containing 25 mL of XAD-2 resin instead of larger columns containing 200 mL resin.
- o the elution of the colorants from the XAD-2 resin with a mixture of ammonia: methanol: water 4:40:56 instead of doing a two step elution with 4% ammonium hydroxide first followed by methanol.

Loading and elution rates were reduced and as a result colour recovery times were reduced to one third of the former method.

It was found that sufficient colour solids were recovered on the Pharmacia C16/20 columns for HPLC studies by treating the following amounts of refinery samples (g sugar solids): raw liquor 200-300 g, fine liquor 600-1000 g and refined sugar (third strike) 2000 g.

HPLC Analysis

Chromatographic system. The HPLC apparatus consisted of a high pressure solvent delivery system model 6000A (Waters Assoc), model U6K injection system (Waters Assoc), model M450 variable wavelength UV detector (Waters Assoc); Omniscrite two pen recorder (Houston Instruments) and a Systems 1 computing integrator (Spectra-Physics). A second pump, a model M45 solvent delivery system (Waters Assoc) and a model 660 solvent flow system programmer (Waters Assoc) were connected to the unit. Later in the studies the chromatographic system was upgraded by replacing the recorder and integrator with a model 730 Data Module (Waters Assoc) and the variable wavelength UV detector was replaced with a fixed wavelength model M440 detector (Waters Assoc).

Columns. A number of HPLC columns were evaluated for their resolution of raw sugar colorants. A μ Bondapak phenyl steel column, 4mm ID x 300mm length (Waters Assoc), was finally selected as a compromise between the resolution of the colour components and the time required for a separation. Another column had superior resolution but the time taken for one run was prohibitive.

Chemicals. Gradient elution was used employing the same solvents as Strack et al. (1979) for the separation of flavones: Solvent A methanol: acetic acid: water 5:5:90
Solvent B methanol: acetic acid: water 90:5:5
Methanol and acetic acid were HPLC grade; distilled water was purified by passing through a Milli-Q water system or by redistilling in all glass apparatus.

Sample preparation. About 10 mg colour solid was dissolved in sufficient solvent to give a 2% solution and filtered through a 0.45 μ m filter (Millipore HATF). Samples size for HPLC was 25 μ L.

Procedure. The chromatograph was operated at ambient temperature with the following conditions: flow rate 2.0 mL/min; detection 365 nm; sensitivity 0.04 a.u.s (absorbance units full scale) with the variable wavelength detector and 0.05 a.u.s with the fixed wavelength detector; recorder chart speed 0.5 cm/min; retention times were printed out by the integrator or data module. The elution process was as follows: 15 minutes of solvent A, then a linear gradient from 0 \rightarrow 50% B over 50 minutes. Total time for one run was about 105 minutes after washing the column and reversing the gradient.

Identification of HPLC peaks. The peaks of the HPLC colorant profile of raw sugar colour solids were identified by thin layer chromatography (TLC). A large sample was injected on to the HPLC column and fractions were collected which corresponded

to the peaks. The components in each fraction were equated to a particular spot or spots on a chromatogram of the raw sugar colorants, following conventional methods for flavonoids reported earlier (Linecar et al. 1978). Some fractions were subjected to electrophoresis at pH 2.0 to confirm the presence of tricin 7-glucoside sulphate (T7GS). The composition of some peaks of raw liquors, fine liquors and the corresponding granulated sugars was also checked in the same ways.

The retention time of a few reference flavonoids and phenolic acids was determined as a guide to the order of elution by HPLC. The compounds used were vitexin, apigenin 7-glucoside, apigenin, chlorogenic acid and ferulic acids from commercial sources and tricin 7-diglucoside isolated in CSR Research Laboratories (Linecar et al. 1978).

An HPLC chromatogram was also run on a solution of polymeric colorant of factory origin, which was isolated from a sugar process sample by the method reported earlier (Linecar et al. 1978). This colorant was defined as the fraction excluded from Sephadex LH-20 and in this sample accounted for nearly half of the colour solids by weight.

Standard flavonoid. Apigenin, a flavone aglycone, was used as a standard because many of the flavonoids found in cane sugar are flavones, being derivatives of apigenin and tricin. In early trials apigenin was used as an external standard to estimate the concentration of colorants in the original sugar sample. A calibration graph was obtained of peak height versus amount of apigenin over a range of concentrations, keeping the injection volume constant. Then all peak heights on the HPLC profile were related to the peak height of a known amount of apigenin. In the last trial, where carbon was the decoloriser, apigenin was used as an internal standard. That is a known amount of apigenin was added to a known amount of sample prior to injection.

When the same weight of factory produced colorant, phenolic compounds such as ferulic acid, and flavonoids was injected on to the column the peak height or response was quite different. The response of several flavonoid compounds was of similar order to apigenin e.g. 75-115 per cent of apigenin, and therefore it was assumed that sugarcane flavonoids could be estimated approximately using apigenin as standard. The peak height of factory colorant was much less than apigenin and it would be grossly underestimated using this standard. The response of phenolics, 10-20 per cent of that of apigenin, was such that they would also be underestimated but not to the same extent as factory colorant.

Factory Trials

The data used in this paper were obtained from four selected trials in a series of refinery trials in which three different decolorisers were used to produce fine liquor. Trials A and B were conducted at two refineries which had conventional bone char stations. Trial C was done at a refinery using strong base anion exchange resin as the sole decoloriser. The operation of this decolorising system was reported earlier (Cunneen and Hawkins 1972). Trial D was conducted at a refinery in which granular carbon and char stations operated in parallel with two-thirds of the refinery melt being treated by the carbon decoloriser. The carbon station consisted of two pairs of fixed beds operating in series.

Weekly composites of refinery raw liquors, fine liquors and corresponding third strike granulated sugar, designated as RL, AFL and 3A respectively in the tables, were sampled in the above refineries. The colour attenuation at pH9 and the indicator value (colour at pH9 divided by colour at pH4) of these samples are listed in table 1. The colour of the granulated sugar at natural pH is also given in this table. The darker grade of refined sugar was used so that sufficient colour solids could be recovered in a reasonable time. Refined sugar was not available from fine liquor decolorised by granular carbon alone, as the refinery used bone char and carbon in parallel for decolorisation.

RESULTS

HPLC Analysis

HPLC colorant profiles. When the HPLC pattern became evident, peaks on the profiles were numbered in the order of elution from 1 to 48. Figure 1 shows many of the peaks or distinct shoulders in an HPLC colorant profile of an affined sugar. The colorants were eluted in order of decreasing polarity with the more polar compounds such as polymeric colour and complex phenolics being eluted before the less polar flavonoid colorants. Peak 1 contained a portion of the factory colorant, peaks 2-16 contained simple phenolic acids such as ferulic acid or more complex derivatives, and peaks 17-48 contained flavonoid compounds. The major flavonoid peaks in raw liquor were usually 21 and 41 the main constituents of which were apigenin glycosides (possibly di-C-glycosides) and tricin 7-diglucoside (T7GG) respectively. Another characteristic peak was 34 which contained tricin 7-glucoside sulphate (T7GS).

Estimation of colorants by HPLC. As will be observed this colorant profile and others did not have a horizontal base line. This was a consequence of factors such as gradient elution and the amount of factory colorant in the sample.

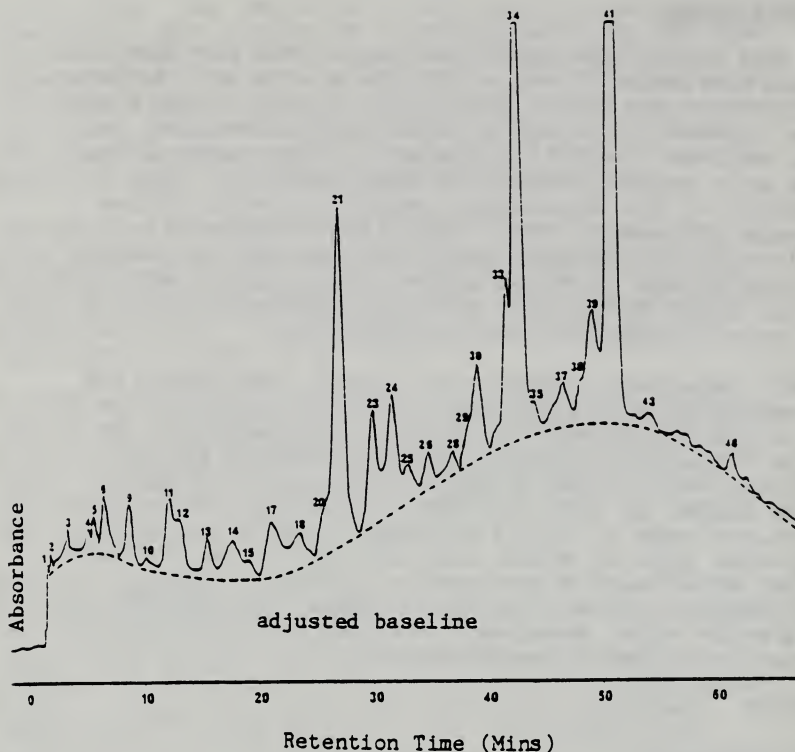


Figure 1.--HPLC profile of cane sugar colorants ex affined sugar. Sensitivity 0.04 aufs

The HPLC profile of this colorant is shown in figure 2 and it indicated that this class of colorant was eluted throughout the HPLC separation. As removal of this colorant would be extremely time consuming a base line correction was applied as shown by the dotted line in figure 1. As a check on the quantitative aspects known amounts of apigenin were added to samples of colour solids and satisfactory recovery was obtained using the adjusted base line as presented in table 2.

The concentration of flavonoid colorants was calculated relative to apigenin and expressed as ppm on sugar solids in the original sample. This was done for each peak which was greater than 0.5 cm at 0.04 aufs when measured from the adjusted base line, using the peak height of the colorant, the peak height and concentration of apigenin, and the per cent colour solids (i.e. grams colour solids recovered from

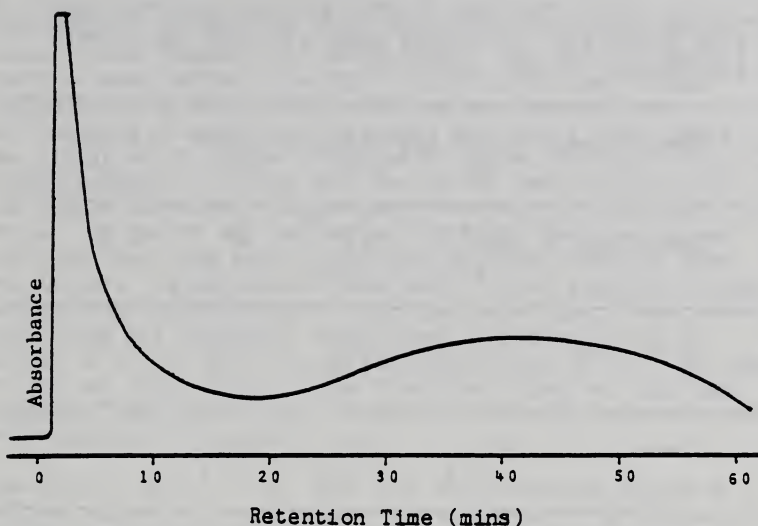


Figure 2.—HPLC profile of polymeric colorant.
Sensitivity 0.05 aufs

100 grams sugar solids). It was assumed that recovery of colour solids from each sample was complete and that the response of all flavonoid colorants and apigenin was the same.

The concentration of factory colorant and phenolic derivatives was not calculated because of the lower response of these colorants compared to apigenin.

The relative concentration of flavonoid colorants in ppm on sugar solids in raw liquor and fine liquor is given in the Appendix; in table 3 for bone char refineries (Trials A and B), in table 4 for the resin refinery (Trial C) and in table 5 for the granular carbon/char refinery (Trial D). The results for refined sugar (3rd strike) have also been included in the above tables where applicable.

The HPLC profiles of colorants of raw liquor before and after bone char, resin and granular carbon decolorisation for given trials are shown in figures 3 to 5 respectively. The profiles of colorants in fine liquor and the corresponding 3A sugar are shown in figure 6.

Removal of Colorant Types

Previous laboratory and factory colour investigations based

Table 1.--Colour and indicator value of raw liquor, average fine liquor and 3A refined sugar for bone char, resin and granular carbon refineries

Trial No	Decoloriser	Colour and RL	Indicator Value AFL	3A	Colour 3A Natural pH
A	Bone Char	1280 [#] 2.7	163 1.9	50 1.8	28
B	Bone Char	1180 2.2	143 1.6	48 1.5	45
C	Resin	1160 3.3	227 3.4	61 2.3	40
D	Carbon	1460 3.8	130 1.7	not determined	
D	Bone Char	1460 3.8	302 2.8	not determined	

Colour (pH9) of the sample is the upper value and indicator value is below it in each set of results.

$$\text{Colour} = \frac{1000 \times \text{absorbance } 420 \text{ nm}}{\text{cell size (cm)} \times \text{concentration (g/mL)}}$$

$$\text{Indicator value} = \frac{\text{colour at pH 9}}{\text{colour at pH 4}}$$

Table 2.--Check on recovery of apigenin in HPLC analysis of colorants

Amount added μg	% Recovery
1.08	93
1.25	90 -104
1.5	100-103
1.92	90 -106
2.85	94 -104

on conventional colour measurements indicated that decolorisers differed in their propensity to remove certain colour types (Kennedy and Smith 1976). The data in table 1 confirm these earlier findings. It is clear that in CSR refineries bone char and granular carbon have a higher affinity for pH-sensitive colorants than resin since there was little or no change in indicator value following resin decolorisation. In bone char and carbon decolorisation of raw liquor there was a significant decrease in indicator value. In one of our refineries (Trial D) granular carbon and bone char were used in parallel to decolorise the same raw liquor. It is evident from table 1 that granular carbon removed more pH-sensitive colour than bone char. When this raw liquor was partially decolorised by carbon it had a colour of 270 at pH 9 and an indicator value of 2.1. As this colour was only slightly less than fine liquor ex char this suggested that carbon had a higher affinity for pH-sensitive colorants than bone char.

The data in table 1 also show that the indicator values of refined sugar reflected those of fine liquor in Trials A and B, i.e. the colorant composition of refined sugar was related to that of fine liquor. In Trial C with a higher proportion of pH-sensitive colorants in fine liquor some of these colorants were removed in the crystallisation step. Also more pH-insensitive colorants may have been formed in sugar boiling thus lowering the indicator value of the sugar.

The application of HPLC to factory colour studies has enabled us to obtain more details of the changes in colorant composition following the decolorisation of raw liquor with bone char, resin and granular carbon than with other techniques such as gel filtration.

Bone Char Decolorisation

The HPLC colorant profiles of raw and fine liquor of Trial B in figure 3, show that bone char decolorisation resulted in the removal of many of the flavonoid peaks, in particular peaks 33-48 which included the tricin derivatives T7GS and T7GG. The apigenin derivatives were the main flavonoid pigments found in fine liquor ex bone char as shown by peak 21 in figure 3 and confirmed by TLC.

From the profiles in figure 3 it is evident that the peak corresponding to part of the factory colorant increased considerably in fine liquor in Trial B. Some phenolic colorants were also removed and other phenolic peaks were smaller, such as peak 13.

The results in table 3 indicate that at least 95 per cent of the total flavonoid concentration was removed by bone char decolorisation in these trials. However up to 15 per cent of

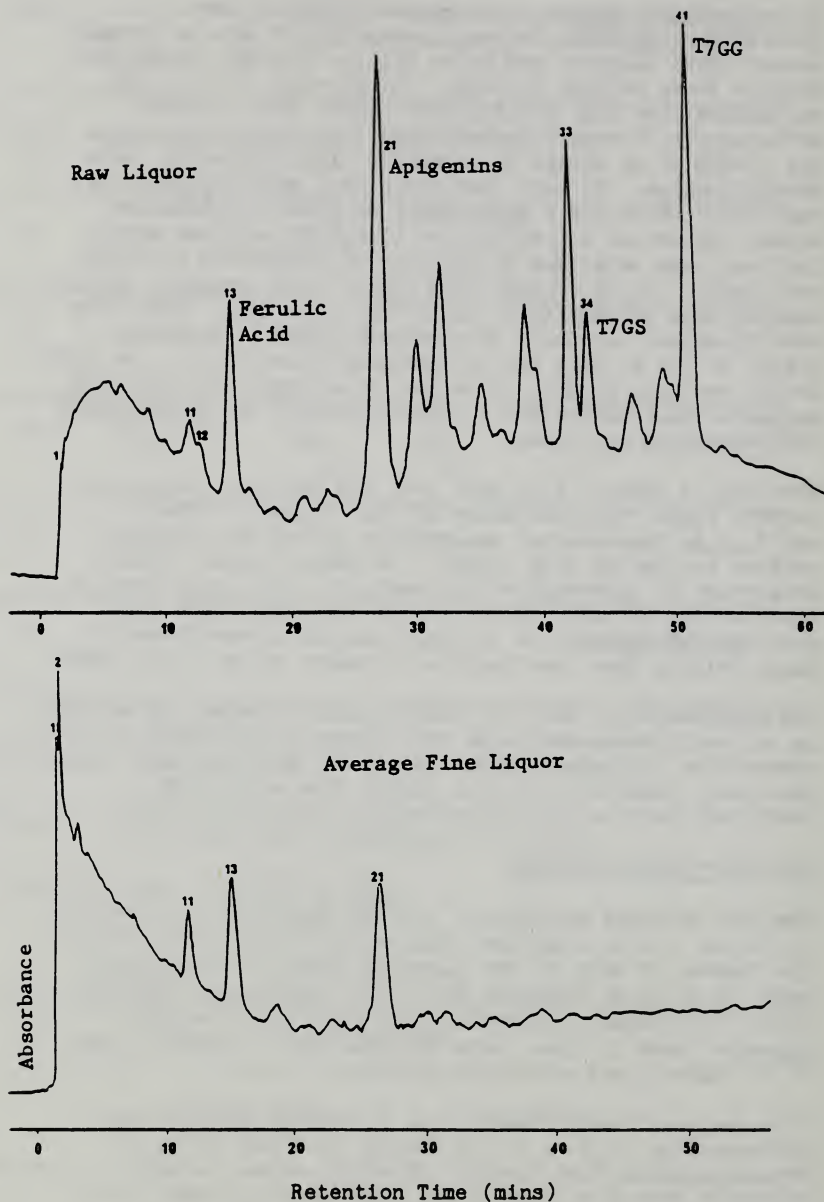


Figure 3.--HPLC Profiles of colorants in raw and fine liquor from a bone char refinery - Trial B. Fine liquor sample size x 6.13 of raw liquor. Sensitivity 0.04 aufs

the apigenin derivatives in raw liquor was not removed by char.

The low indicator values of the fine liquor ex bone char reflected the removal of flavonoids from raw liquor.

Resin Decolorisation

The data in table 4 show that only a few flavonoid peaks were removed completely by resin decolorisation, such as T7GS, peak 34. However the HPLC profiles in figure 4 show that while some flavonoid peaks in fine liquor ex resin were smaller than in raw liquor e.g. peak 21, apigenin derivatives, and peak 41, T7GG, others were taller such as peaks 24, 26, 29, 33 and 39. It should be noted that peaks 26, 29 and 39 were minor peaks in raw liquor thus indicating that the components of these peaks were far less readily removed than the major flavonoids.

From TLC analysis it was found that the apigenin glycosides, dominant components of peak 21 in raw liquor, were quite weak in fine liquor ex resin. The principal components of this peak in fine liquor were two compounds which were minor constituents of peak 21 in raw liquor. This indicated that the apigenin derivatives were removed more efficiently than these minor compounds. The main peak in fine liquor ex resin, peak 33, had a major constituent which could be an apigenin mono-C-glycoside from its TLC and HPLC properties.

From table 4 it is evident that resin removed about 70 per cent of the total flavonoid concentration but almost 90 per cent of peak 21 and 80 per cent of T7GG. However the concentration of peaks 26 and 29 was the same in raw and fine liquor and that of peak 33 only slightly lower in fine liquor.

The raw and fine liquor had the same high indicator value; this was consistent with the high proportion of flavonoids in both feed and resin decolorised liquors.

Granular Carbon Decolorisation

The HPLC profiles in figure 5 and data in table 5 show that granular carbon was an efficient decoloriser. It removed all flavonoid colorants as no peaks were detected for these compounds in fine liquor. The same profile also shows that carbon removed almost all phenolic derivatives.

Figure 5 and table 5 also show that in Trial D bone char was not nearly as effective a decoloriser as granular carbon. Nor was char as effective in Trial D as in Trials A and B. Only 85 per cent of the total flavonoid content was removed by char in Trial D. The apigenin derivatives in peak 21 were

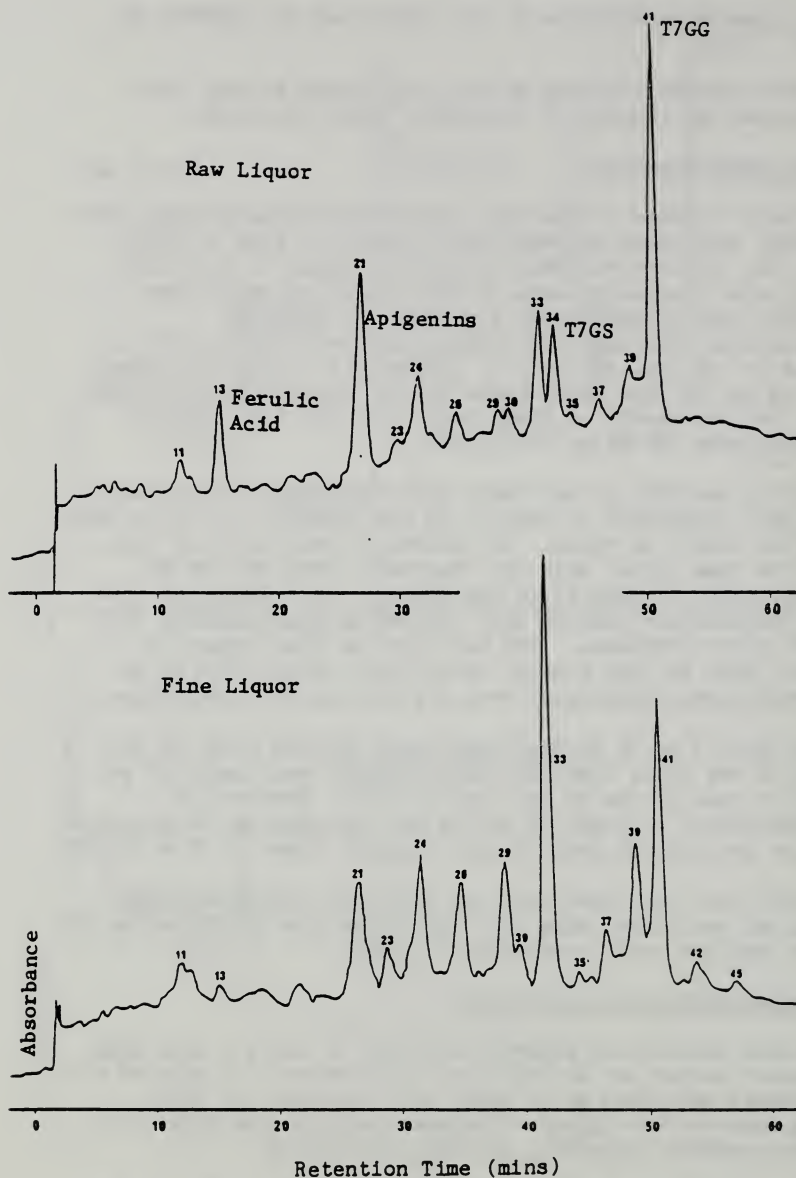


Figure 4.--HPLC profiles of colorants in raw liquor and fine liquor from a resin refinery - Trial C. Fine liquor sample size x 4.15 raw liquor. Sensitivity 0.04 aufs

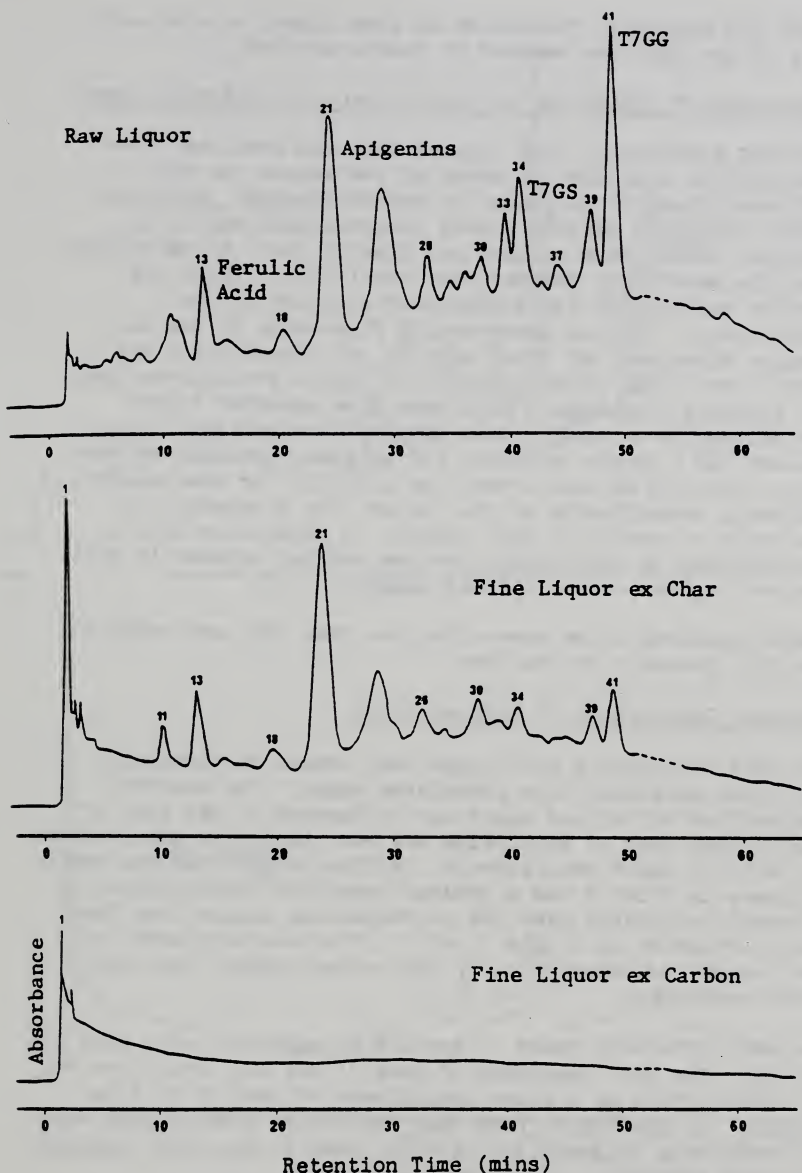


Figure 5.--HPLC Profiles of colorants in raw and fine liquor from a refinery with bone char and carbon decolorisation in parallel - Trial D. Fine liquor sample size: ex char x 3.7 raw liquor, ex carbon x 8.9 raw liquor. Sensitivity 0.05 aufs. Internal standard peak omitted.

again the strongest flavonoids in fine liquor ex char and only 75 per cent was removed by decolorisation.

Comparison of Affinities of Decolorisers for Colorant Types

The HPLC profiles of fine liquors obtained from the three decolorisers indicated an order of preference for the different types of colorants by the adsorbents. In these trials flavonoid colorants were adsorbed most easily by granular carbon then by char and finally resin as calculated from the percentage removed from raw liquor. There was further selectivity for different flavonoids by the decolorisers. Carbon adsorbed all flavonoids in the one example discussed but other work in our laboratories has shown that it has more affinity for tricetin derivatives than for apigenin compounds. Bone char also adsorbed tricetin derivatives more readily than apigenin derivatives. Resin however had a higher affinity for apigenin derivatives than tricetin derivatives and a very low affinity for some minor flavonoid constituents of raw liquor. The presence of flavonoid colorants in fine liquor, in particular tricetin derivatives, is detrimental for the refiner because it will lead to higher colored refined sugars.

Carbon appeared to be more effective than char and resin for removing phenolic derivatives.

Colorant Composition of Refined Sugar

The data in tables 3 and 4 show that traces of flavonoid colorants persisted into granulated sugar. The colorant composition of refined sugar was influenced by the type of decoloriser used to decolorise the raw liquors as may be seen in tables 3 and 4 and figure 6. Refined sugar from the resin refinery in Trial C had a greater range and concentration of flavonoid colorants than the corresponding sugars from bone char refineries in Trials A and B. This was consistent with the higher indicator value of the refined sugar from the resin refinery.

The most prominent peaks in granulated sugar ex resin were 31 and 33. The main component of peak 31 was not identified but it was observed as a minor constituent of peak 30 in fine liquor. In the sugars from char refineries a major peak was 21 containing apigenin derivatives; peak 31 was also detected in these samples.

The HPLC profile of refined sugar in figure 6 also shows an increase in peak 1 compared to fine liquor indicating formation of factory colorant in crystallisation; this would lower the indicator value of the sugar.

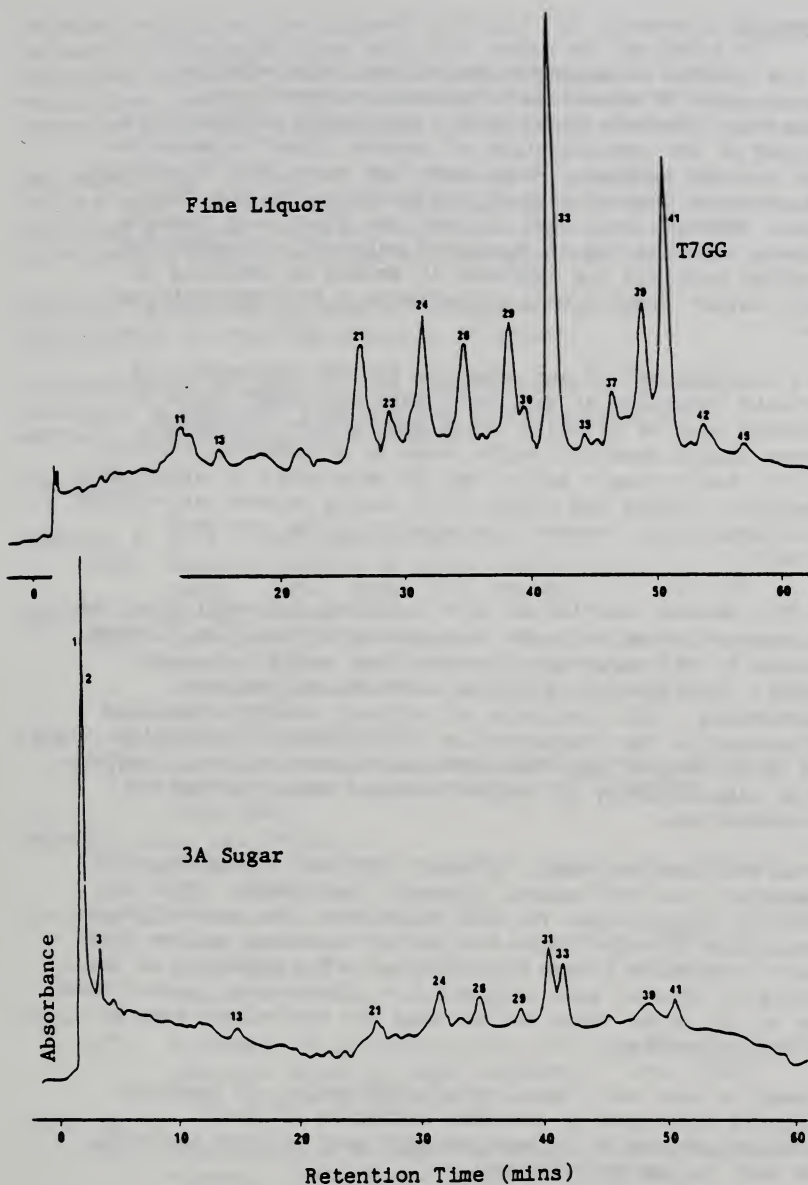


Figure 6.--HPLC Profiles of colorants in fine liquor and 3A sugar from a resin refinery - Trial C. Sugar sample size x 2.55 fine liquor. Sensitivity 0.04 aufs

DISCUSSION

The use of HPLC to separate and estimate the relative concentration of cane sugar flavonoid colorants was successful. For the first time a reasonable estimate was obtained of the concentration of natural plant pigments in sugar process samples. This study has shown that flavonoid colorants are present only at ppm level in raw and fine liquor. However this work is just the first step towards accurate measurements of flavonoid colorant concentration. As column packings are improved it should be possible to obtain better resolution combined with a shorter analysis time.

While the HPLC method was adequate for the estimation of flavonoid colorants it was unsuitable for other types of colorants such as phenolic acids and factory colorants. However simple phenolic acids, such as p-coumaric, ferulic, vanillic and syringic acids, may be determined by other HPLC techniques (Curtin and Paton 1980), using solvent extraction to preferentially recover the phenolic acids for HPLC analysis.

The HPLC method contributed more detailed information on the differences in the colorant composition of the fine liquors produced by the three decolorisers than would have been possible with gel filtration or conventional colour measurements. HPLC analysis of refinery products detected differences in the concentration of individual flavonoids. Thus while samples may have the same colour at pH 9, they may differ significantly in their flavonoid constituents and concentrations.

Carbon and bone char had a greater affinity for the more intensively colored natural pigments than resin. This had practical implications for the refiner in that the colorant composition of white sugar was partly dependent on the fine liquor from which it was crystallised. The presence of small amounts of natural cane pigments i.e. flavonoids, contributes more to the white sugar colour than the equivalent mass of factory colorants.

We need to have more detailed identification of the cane flavonoid colorants if reliable estimates of their concentration are to be made in mill and refinery products. This work is now in progress.

CONCLUSIONS

The application of colour concentration and HPLC techniques to colour studies in CSR refineries has shown that bone char, resin and granular carbon have characteristic preferences for the removal of colorants from raw liquor.

Granular carbon had the highest affinity for flavonoid colorants followed by bone char then resin on the basis of percentage removal. Carbon adsorbed apigenin and tricin derivatives readily; char showed a preference for tricin compounds and resin a preference for apigenin derivatives.

The decoloriser influenced the colorant composition of refined sugar in that sugars crystallised from fine liquor ex resin had a higher proportion of flavonoid colorants than corresponding sugars produced at bone char refineries.

Granular carbon was the most efficient of the three decolorisers in removing phenolic colorants.

An approximate estimate of the concentration of flavonoid colorants in refinery products was obtained using HPLC analysis with apigenin as a standard.

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Appendix

Table 3.--Relative concentration[#] of flavonoid colorants in raw liquor, average fine liquor and 3A refined sugar from bone char refineries

Peak No	Components	Trial A			Trial B		
		RL ppm	AFL ppm	3A ppm	RL ppm	AFL ppm	3A ppm
16							
17		0.1			0.04		
18		0.1			0.04	0.01	
19							
20							
21	Apig. deriv.	0.4	0.05	0.005	0.6	0.03	0.005
22							
23		0.1		0.005	0.2	0.01	0.003
24		0.1	0.02		0.3	0.01	
25		0.1			0.04		
26		0.1	0.02		0.08	0.01	0.003
27					0.04		
28							
29		0.1			0.2		
30	Tricin deriv.	0.1	0.02		0.1	0.01	
31				0.005			
32		0.1	0.02				
33		0.2			0.4		
34	T7GS	0.1			0.2		
35		0.1					
36							
37		0.1			0.08		
38							
39		0.1			0.1		
40		0.1			0.04		
41	T7GG	0.4			0.6		
42					0.04		
Total		2.4	0.13	0.015	3.1	0.08	0.011

[#] The concentration of colorants was calculated using apigenin as an external standard and assuming the same response factor for all compounds.

Table 4.--Relative concentration[#] of flavonoid colorants in raw liquor, average fine liquor and 3A refined sugar from a resin refinery

Peak No	Components	RL ppm	Trial C AFL ppm	3A ppm
16				
17		0.1	0.03	
18		0.1		
19				
20				
21	Apig. deriv.	0.8	0.1	0.01
22			0.03	
23		0.1	0.03	
24		0.3	0.1	0.01
25		0.1		
26		0.1	0.1	0.01
27				
28				
29		0.1	0.1	0.01
30	Tricin deriv.	0.1	0.03	
31				0.03
32				
33		0.5	0.4	0.02
34	T7GS	0.4		
35		0.1	0.03	
36			0.03	0.01
37		0.1	0.05	
38			0.03	
39		0.2	0.1	0.01
40		0.1		
41	T7GG	1.5	0.3	0.01
42			0.03	
43				
44				
45			0.03	
Total		4.7	1.5	0.12

[#] The concentration of colorants was calculated using apigenin as an external standard and assuming the same response factor for all compounds.

Table 5.--Relative concentration[#] of flavonoid colorants in raw liquor and average fine liquor from a refinery with bone char and carbon decolorisation in parallel - Trial D.

Peak No	Components	RL ppm	AFL ex Char ppm	AFL ex Carbon ppm
16		0.04	0.01	
17				
18		0.1	0.03	
19				
20		0.05		
21	Apig deriv.	0.9	0.22	
22				
23		0.1	0.01	
23a		0.5	0.08	N
24				o
25		0.1	0.02	t
26		0.2	0.04	
27				
28		0.1	0.01	d
29		0.1		e
30	Tricin deriv.	0.1	0.04	t
31				e
32			0.02	c
33		0.3	0.02	t
34	T7GS	0.4	0.03	e
35		0.04	0.01	d
36			0.01	
37		0.1	0.01	
38			0.01	
39		0.3	0.03	
40				
41	T7GG	1.0	0.06	
45		0.02		
46		0.04		
47				
48		0.05		
Total		4.5	0.66	0

[#] The concentration of colorants was calculated using apigenin as an internal standard and assuming the same response factor for all compounds

DISCUSSION

M. A. CLARKE: As you know, several refineries in the U. S. use ion exchange resins in treating their liquors. I think there are some differences in both the process steps and the design of the resin systems from those in Australia. Would you care to discuss this?

P. SMITH: Do these refiners use the resin as the sole decolorizer?

M. A. CLARKE: No, they don't. Also the size of the system relative to melt is rather different, and the design of the columns is different. The resins are different too, of course.

P. SMITH: I suspect that if you had bone char preceeding resin, you would avoid much of this trouble because bone char removes quite a few of these color precursors which resin does not and this would avoid the color formation that takes place. In our plants we use resin as the sole decolorizer.

D. LEIGHTON (International Sugar Journal): The difference between the tricin and apigenin molecule is 2 methoxy groups which are not especially polar or active. The differential adsorption which has been demonstrated indicates therefore that the adsorption must be physical rather than chemical. Would you care to speculate on the reasons for these differences.

P. SMITH: Going back to organic chemistry, these two methoxy groups have inductive effects. Upon a raise in pH, the tricin derative becomes much more easily negatively charged as compared with apigenin. Whether somthing that is more negatively charged goes on by physical adsorption, I would not like to speculate. I suspect that it is a small difference in charge structures at particular pH's that are related to the methoxy groups. These groups do withdraw electrons from nearby in the molecule. The tricin molecule gets more excited as the pH is raised.

C. C. CHOU: Based on your understanding of the color profile and the selectivity of various adsorbents, if you were to build a new decolorization station, what would be your recommendation in regard to the decolorizer or combination of decolorizers.

P. SMITH: There is no perfect decolorizer. Granular carbon removed all colorants, but it is like resin, it does not remove amino nitrogen derivatives, so color is formed. The thing that saves granular carbon is that it is such a good decolorizer, that it can accomodate a small amount of color formation in the decolorized liquor. We could certainly devote a complete symposium to this topic discussing the advantages and disadvantages of each of the decolorizing systems.

F. PARRISH: Have you done control experiments to see to what extent the color formation comes from the adsorbents themselves? Also, to see to what extent these colorants are artifacts?

P. SMITH: We have not gone as far as that, but we feel confident that when we detect a flavonoid, we do in fact have a flavonoid. There is no way that you could form these pigments in process as an artifact. They have characteristic UV absorptions, so they would not be confused. However, I could not comment on other compounds such as polymeric colorants.

B. RAVNO: Is there any indication from the work that you have done that green cane produces different quantities of colorant than burnt cane? Have you noticed any seasonal trends in the quantity of color in raw liquor?

P. SMITH: As yet, in the refinery we can not tell whether the raw sugar came from green or burnt cane or early or late in the season. However, when these techniques are applied to cane juice and to cane leaves, there is no doubt that local conditions and latitude play a part in these pigments.

CRITERIA FOR BONE CHAR EVALUATION

Chung Chi Chou

Amstar Corp.

INTRODUCTION

Bone char is the oldest large scale adsorbent. Although many small more modern sugar refineries have sought to use more efficient decolorizing systems such as phosphatation, carbonatation, activated carbon and auxiliary decolorizing resins, bone char is still the most widely used adsorbent for treating cane sugar liquor. In recent years, a drastic increase in both energy and char stock carrying costs necessitates maximum performance of a char house operation including strict control on the quality of both stock char and incoming new char. This paper presents the analytical methods developed by our laboratory for evaluation of bone char quality. It is interesting to note that although the function of bone char is for decolorization, deashing and buffer capacity, none of these criteria have been included as bone char specifications in commercial purchase contracts.

COLUMN TEST - DECOLORIZATION, DEASHING, BUFFER CAPACITY

The design of a testing procedure for decolorization requires a fundamental understanding of the kinetics of the adsorption process. The mechanism by which sugar colorants are adsorbed onto a carbon surface may be divided into four consecutive steps as illustrated in Figure 1, namely dispersion, interparticle film diffusion, intra-particle diffusion, and adsorption onto surface sites. The selection of the test conditions rests on the knowledge of the relative significance of these sequential steps. The slowest step becomes the rate determining step in the overall rate process.

Under typical refinery practices, diffusion, both inter- and intra- particle, is most likely the determining rate step.

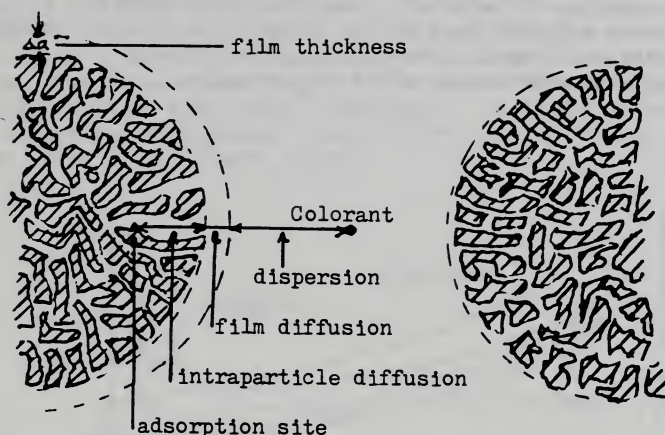


Figure 1.--Adsorption model.

The variables affecting the diffusion may be correlated by a expression derived from the Stokes-Einstein equation as follows:

$$D = F \left(\frac{T}{\mu v^n} \right)$$

where D, the diffusion coefficient, is a function of temperature, T, viscosity, μ , and the molecular size of the colorants. Therefore, the Brix and temperature of a sugar solution at the test conditions would affect the rate of adsorption. Since the decolorization also depends on the driving force, i.e., the colorants concentration in the solution and that immediately adjacent to the carbon surface, it is obvious that the flow rate of the feed liquor in a column test, and the rate of rotation of a test tube in a batch test would also influence the results.

The column test procedure described in Appendix I represents a compromise in test conditions to produce results which would most closely correlate to field experience. In the following study, the quality of new chars is evaluated in terms of their decolorizing characteristics, deashing pattern and buffer capacity.

The percent color removal of several new chars is plotted in Figure 2 as a function of liquor volume displacement. It can be seen that Supplier C char was superior to other new chars evaluated. Supplier A char was somewhat better than Supplier B char in the early stage of the liquor cycle. However, as the feed continued into its extended cycle, the quality of Supplier B char surpassed that of Supplier A char. On the other hand,

although the quality of Supplier D char was considerably inferior to both A and B chars in the early stage of the liquor cycle, the difference in quality between A and D chars diminished in the later stage of the liquor cycle.

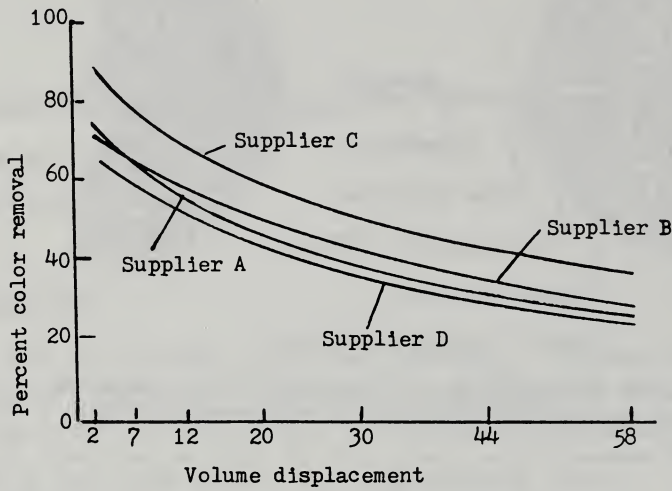


Figure 2.--Laboratory column test, % decolorization of new bone chars, 4th quarter 1980.

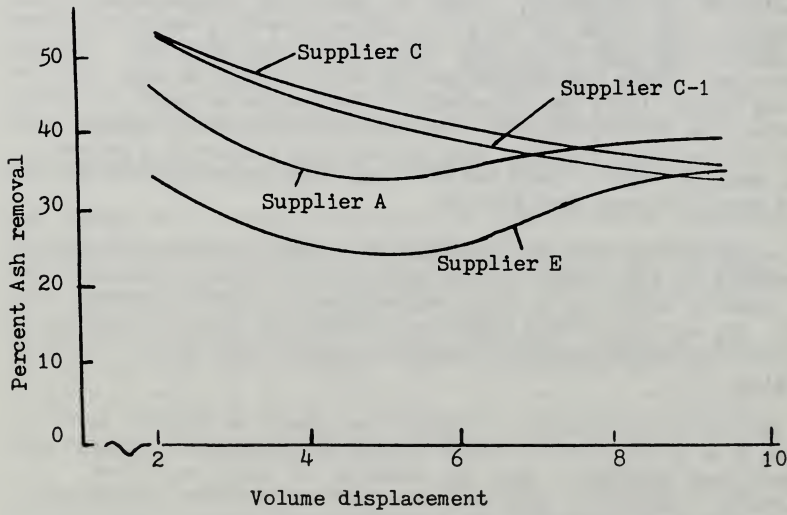


Figure 3.--Laboratory column test, % deashing of new bone chars.

Figure 3 shows the percent deashing pattern of new chars from different countries of origin. It is seen that the deashing capability of Supplier C again performed better than other chars. The percent deashing of chars from different shipments by Supplier C is also rather consistent. It is not known why the % deashing of Supplier A and E chars went up during the later stage of the liquor cycle.

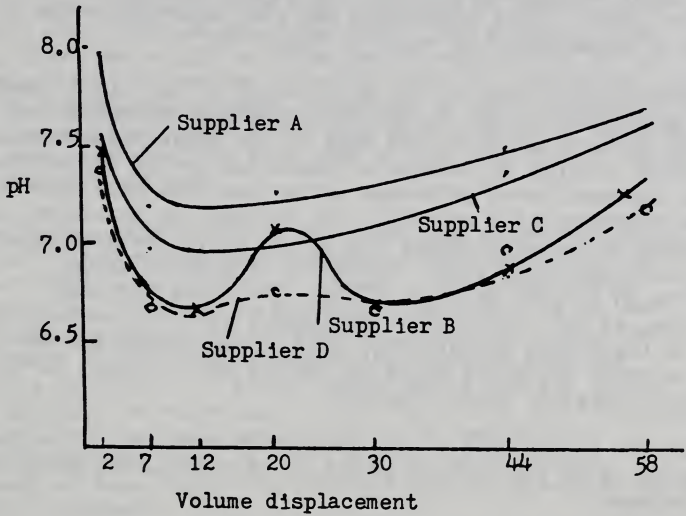


Figure 4.--Laboratory column test, buffer capacity, new bone chars, 4th quarter 1980.

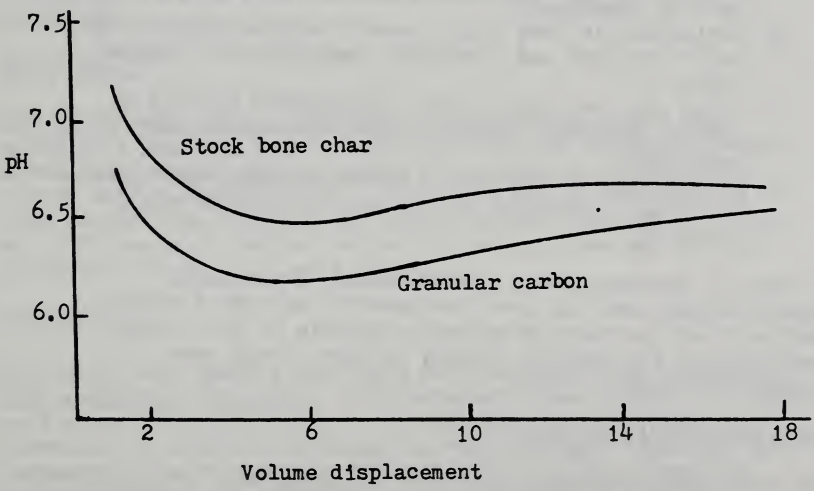


Figure 5.--Laboratory column test, buffer capacity, stock bone char vs granular carbon.

The buffer capacity profile of chars from various suppliers is depicted in Figure 4. It is noted that although % deashing and decolorization of Supplier C char was superior to all other chars, as discussed previously, the buffer capacity of Supplier C char was not as good as that of Supplier A char. A somewhat unexpected finding of the pH profile study was that the pH of liquor effluent from all supplier's chars increased when the liquor cycle was extended beyond the normal char filter operation range. This phenomenon was also observed on the pH profile of stock chars as shown in Figure 5.

The accelerated pH drop in the early stage of the liquor cycle may be attributed either to the replacement of hydronium ion from the carboxyl group on the edge of the basal plane carbon by divalent cations or release of hydronium ion by the acidic anionic colorants when exchanged and/or adsorbed onto the surface hydroxyapatite or both. The increase in hydronium ion concentration in sugar liquor due to the above two phenomena would also auto-catalyze the formation of organic acids resulting in further decrease of pH. Following the same reasoning, as the liquor cycle continued, both the carboxylic group on the carbon and exchange site on hydroxyapatite for acidic anionic colorants would be "exhausted" avoiding further pH drop and organic acid formation.

Although the traditional column decolorization test gives good indication as to the quality of chars, it has the following disadvantages:

1. Considerable time and man power requirements.
2. Lack of sensitivity in measuring quality differences in various adsorbents.
3. Variations in feed liquor make interpretation of the data difficult and long-term comparisons of limited value.
4. Large char and feed liquor requirements as well as special equipment set-ups produced a cumbersome technique.
5. Variability in the final results may have been induced by column channeling and various methods of column settling.

In order to overcome these disadvantages, a new batch decolorization test has been developed to provide a simple, practical and informative procedure capable of evaluating realistically the quality of both refinery stock chars and incoming new chars.

BATCH DECOLORIZATION TEST

For a realistic characterization of any adsorbent system, it is fundamentally important to determine the adsorptive capacity and the rate of adsorption of the test adsorbent. The

classical approach to characterize an adsorption system can be found in the isotherm equations developed by Langmuir and Freundlich. A simplification of this classical approach is the basis for a new batch decolorization test. In the new test the rate of adsorption is a measurement of the color removal by an adsorbent in one hour using 4 grams of char and 20 ml of 30 Brix reference liquor. This above measurement provides the basis for determining the optimum flow rate of sugar liquor through a given char column. Generally, for a typical raw sugar a flow rate of 300 cu ft/hr can be safely used in the early stages of char column operation when the adsorption rate of the test adsorbent exceeds 80 percent of the reference char. When the adsorption rate of the test adsorbent falls below 50 % of the reference char the flow rate of the sugar liquor should not exceed 200 cu ft/hr.

The adsorption capacity of an adsorbent can be defined as the amount of color removed by 1.5 grams of adsorbent using 20 ml of a reference sugar liquor in 4 hours. The color removal capacity of an adsorbent is generally characterized by the total surface area and pore structure of the test adsorbent. The analysis of the rate of adsorption together with the adsorptive capacity of a given system should provide highly useful information regarding the quality of the test adsorbent. In calculating the test results the color removal by a reference char was taken as 100 % and the decolorizing efficiency of each sample was determined as a percentage of the reference adsorbent.

For the interpretation and comparison of test results the following situations are presented together with their analysis.

Situation 1

The batch test shows a high rate of adsorption with a low adsorptive capacity. This adsorbent pictures an active readily accessible surface with possible pore blockage by nonactive carbon and/or inorganic ash. In such a case a reduction in feed liquor flow rate may not significantly increase the overall efficiency of decolorization since the pores may not be accessible to the available colorants.

Situation 2

The batch test shows a high rate of adsorption with an equally high adsorptive capacity. This adsorbent demonstrates a highly active surface with limited pore blockage. For optimum operation it would be necessary to run the sugar liquor at an increased flow rate in the early stages of column operation thereby taking full advantage of the adsorbent's excellent surface capabilities. Later on in this same char cycle the flow rate may be reduced thus allowing the same colorants

sufficient time to diffuse into the internal surfaces of the adsorbent.

Situation 3

The batch test shows a low rate of adsorption and a high adsorptive capacity. This adsorbent demonstrates a relatively inactive, readily accessible surface with a partial blockage of relatively active internal pore surface. In this situation a relatively slow flow rate would tend to utilize the adsorbent's strong point, its active pore surface.

Situation 4

The batch test demonstrates a low rate of adsorption and a low adsorptive capacity. This adsorbent pictures an inactive surface and significant pore blockage. If all efforts to bring this adsorbent back to life have proven unsuccessful it is then a candidate for discard.

The following are some advantages of the batch decolorization test:

1. Considerable savings in time and man power. In general for a quarterly char survey, the time required is about one week for the batch test versus three to four weeks for the column test.
2. Long term comparisons are possible if a reference liquor is used for the test. In developing the test, a press filtered washed sugar liquor (Brooklyn) and a reference sugar liquor made from an Australian raw sugar were used.
3. The batch test provides more useful information which lends itself well to a realistic evaluation of adsorbent quality and suggests possible corrective action leading to adsorbent improvement and better maintenance. These parameters are also useful in determining optimum char filter operation.

For those who are interested in this char quality control tool, the procedure is attached as Appendix II.

The batch decolorization test was incorporated into a routine quality control program in which quarterly surveys of the quality of stock char of each refinery were made. The quarterly quality data are used as a diagnostic tool and guidance for appropriate action taken to keep the char quality consistently at an acceptable level. Before the implementation of this batch test method, the quarterly batch decolorization data of a refinery stock char were compared to the quarterly plant decolorization experience over a period of one year. The two results correlated surprisingly well as illustrated in Figure 6.

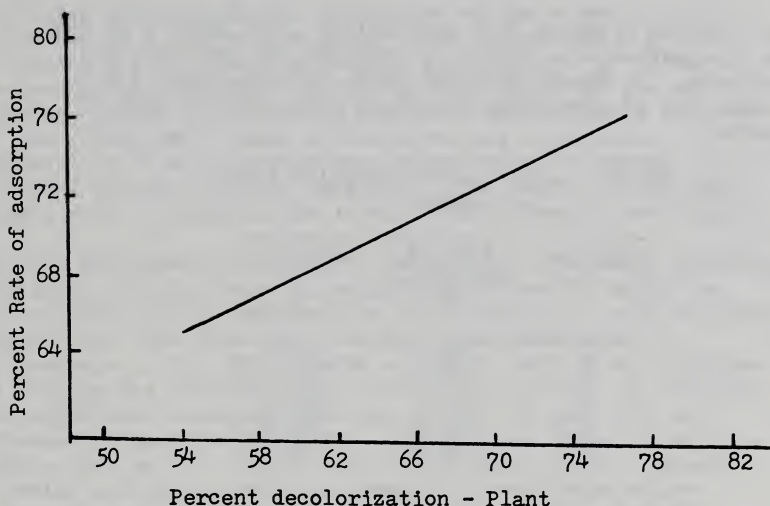


Figure 6.--Batch decolorization test vs. plant experience.

The quality of various stock and discard chars as measured by the batch decolorization test is shown in Table 1. Stock char B represents the first set of a refinery with a two set char system. The quality of this set char was considerably higher than that of the other two stock chars A and C. To be economical, the rate of adsorption for a discard char should be below 30 %. It can also be expected, as illustrated in Table 1, that the capacity of a stock char is always lower than the rate of adsorption. This is mostly due to the blockage of pores by adsorbed impurities and/or inactive carbon resulting from incomplete regeneration of stock chars.

Table 1.--Batch decolorization test, stock bone chars.

Stock Bone Char	Rate of Adsorption %	Capacity %
A	60.0	52.9
B	88.8	88.1
C	51.2	46.5
Discard Char	29.4	24.6

The batch decolorization test has also been used to evaluate the quality of incoming new chars. Table 2 shows the variations in the quality of new chars from several suppliers or potential suppliers. The difference in quality from the

same Supplier C was as high as 6 points. The quality of new char D submitted by a potential supplier was obviously not acceptable. It should be noted that, for new chars, the percent rate of adsorption should be very close to that of capacity.

Table 2.--Batch decolorization test,
new chars.

Supplier	Rate of Adsorption %	Capacity %
A	92.6	89.9
B	91.4	87.4
C	102.1	103.4
C-1	95.8	94.8
D	75.0	75.5

It has been suggested that the decolorization capacity of a new char is inversely related to its bulk density. Table 3 clearly indicates that other factors, such as quality of carbon and porosity of adsorbents, play a role in the decolorization. For the four suppliers tested, while the decolorization of Supplier B char was over 92 % with a bulk density of 41.1, the Supplier D char had the lowest decolorization with a bulk density of 37.9 lb/cu ft. It is interesting to note that the quality of the char of Supplier C is better than that of the reference char used as the control.

Table 3.--Batch decolorization test,
New bone chars - 4th quarter 1980.

Supplier	Bulk Density lb/cu ft	Rate of Adsorption %	Capacity %
A	39.7	87.0	86.5
B	41.1	92.6	92.7
C	35.5	102.1	103.4
D	37.9	77.2	76.9

It should be pointed out that the results of the column test correlate fairly well with those of the batch test.

BONE CHAR DUST ATTRITION TEST

Over the past several years, Dr. V. R. Deitz of the Naval Research Laboratory, Washington, DC, has developed a procedure for the determination of Dust Attrition (DA) of granular activated carbon. For the purpose of this test the dust

attrition coefficient, DA, is defined as the weight of dust collected on a pre-weighed glass fiber filter pad in a vibrating device with a consistent acceleration "g" during a designated time per known weight of adsorbent. An air stream passing through the vibrating sample is used to carry the dust to the filter pad. Under actual operating conditions, degradation of an adsorbent can be attributed to impact force, crushing and attrition or abrasion. The latter is the most common cause of degradation in bone char transport and kilning.

The two published procedures to measure the mechanical handling resistance of bone char typically produce results which are either difficult to interpret or do not correlate with refinery experience. The T-bar abrasion test described in the Proceedings of the 12th Session of ICUMSA determines attrition as long as the particle size is relatively small. In addition, our experience indicates that the results of this test are affected by particle geometry. The ball pan hardness test, as pointed out by Dr. Deitz, applies all three forces (impact, crushing and abrasion) to the sample in a variable manner determined by the size, shape and density of the particle.

The method submitted by Dr. Deitz as "Proposed ASTM Standard Method of Test for Dusting Attrition of Granular Activated Carbon" measures the effect of attrition forces between particles.

This method has been subjected to a collaborative study sponsored by the D-27 committee of the ASTM. The American Sugar Division of Amstar Corporation has participated in this program. We have tentatively adopted this test method for monitoring all incoming new chars. The following Table 4 shows some of these data.

Table 4.--Bone char attrition.

Sample	Dust formation mg/min	Initial dust mg/50 ml
1	1.63	15
2	2.71	230
3	2.59	186
4	0.78	50
5	2.22	82
6	2.23	84
7	1.15	63
8	0.57	64
9	2.21	240
10	2.62	227
11	1.37	48
12	3.27	76

The results clearly indicate the variation in different suppliers with respect to attrition resistance of bone char, particularly in terms of "initial dust" collected. High initial dust obviously increases the cost per shipped weight.

This method has simplicity and is relatively economical in laboratory manning. Presumably this procedure would be adopted by the ASTM.

CONCLUSION

Close monitoring of both stock and new char quality together with adequate control of operating procedures to improve char quality should increase the adsorptive capacity of adsorbents.

With good char quality in stock and through optimization of liquor cycles, the energy consumption and stock char carrying cost in a char house can be reduced to a manageable minimum.

APPENDIX I

Column Test

I. Equipment:

Technicon Auto-Analyser Proportioning Pump
Fraction Collector _ Rotator Type 3401 B
Hot Water Circulator - Bath - Lauda K2
Glass - Pyrex Columns (4), Jacketed, 1" ID, 8" long
Auto-Analyser Pump Tubes 0.4 ml/min
Auto-Analyser Pump Tubes 1.2 ml/min
Spectrophotometer
pH meter
Test Tubes

II. Washing of New Chars Before Decolorization:

Four pre-heated jacketed glass columns are settled in hot water with 80 cc each of representative char*. The 80 cc sample is also weighed in order to express results on a weight basis if necessary. The temperature of the columns is maintained at 80° C by circulating hot water through a constant temperature water bath. Distilled water is introduced downward via Technicon pump at the flow rate of 0.4 ml/min for approx 18 hours. Effluent is collected in clean flasks for conductivity measurements if necessary.

* Place glass wool at each end of column. This washing procedure does not apply to stock bone chars.

III. Feed Liquor Preparation:

Desired feed may be obtained from a refinery if the sugar being processed is suitable for the test. The syrup must be press filtered, and adjusted to 60 - 62 Brix and about 8 pH.

On the other hand the desired feed may be prepared in the laboratory using a reference raw sugar. The prepared syrup must be filtered with Buchner funnels using Johns-Manville Standard Super-Cel #202 Reeve Angel filter paper, and adjusted to 60 -62 Brix and about 8 pH.

IV. Decolorization

Introduce the sugar liquor feed to the columns upward via a technicon pump at a flow rate of 1.2 ml/min for approx. 17 hours or longer if desired. The effluent is collected by an automatic fraction collector at 20 min intervals in test tubes representing about 23.5 ml each. Discard first 5 fractions, and make composite samples of every five fractions of the remaining effluent. Each or every other composite sample, together with the feed liquor are then to be analysed for color, ash and pH.

APPENDIX II

Batch Test

I. Equipment

- Constant temperature oven $\pm 1^{\circ}$ C at 75° C.
- Constant speed end to end shaker - rotator, 10 - 20 RPM (14 RPM)
- Test tube with screw cap, 20 mm OD, 125 mm length, Corning #9825
- Teflon tape - thread sealant, to prevent leakage
- Vacuum pump
- Filtering flask, 1000 ml
- Millipore Filter Apparatus - Funnel
- Millipore Filter Membranes - white, plain, 47 mm, HA, 0.45 μ , with adsorbent pad
- Test tube, 29 mm OD, 200 mm long to fit inside the filtering flask
- Spectrophotometer
- pH meter
- Jacketed column - 1" ID 1 ft long
- Hot water circulating bath
- Technicon Auto-Analyzer Proportioning Pump

All new bone chars have to be pre-washed for the Batch Decolorization Test. The process - or service bone chars are to be used as is - without prewashing.

II. Preparation of "Washed reference new char" and all other new bone char samples

1. A jacketed column, partially filled with water is settled with approximately 50 grams of representative sample.
2. Wash the char column with distilled water at 80° C overnight (16 hours) at a flow rate of about 0.42 ml/min using Technicon Auto-Analyzer Proportioning Pump.
3. Dry the washed char at 105° C overnight.

III. Preparation of reference sugar liquor:

1. Prepare a needed quantity of approximately 31 Brix sugar solution using an appropriate raw sugar.
2. Add 5% by weight of Johns-Manville "Fiber-Cel 7F" (filter aid) to the solution. Mix well.
3. Filter the sugar solution using the Millipore Filter setup with two adsorbent pads and vacuum pump. Rinse flask with first 50 ml.
4. Adjust the filtered sugar solution to 30 Brix with clear distilled water.
5. Read the percent transmittance of the solution at 420 nm in 1 cm cell in a spectrophotometer; it should be approximately 14 % transmittance. (If necessary adjust with a 30 Brix colorless sugar solution to obtain the proper transmittance.)

IV. Procedure:

1. For each Rate of Adsorption weigh out a 4 gram sample. For each Adsorptive Capacity weigh out 1.5 grams. The char must be riffled and be representative of the lot. For each run include reference char.
2. Place each sample in a test tube with screw cap, with help of a paper funnel; apply some teflon tape to threaded area - do not close.
3. Pour 20 ml of reference sugar solution into a 25 ml size graduated cylinder - adjust meniscus with help of an eye dropper. For each run include a Blank.
4. Preheat both the char test tubes (open) and the sugar liquor in the oven at 75° C for 30 min.
5. Add 20 ml of the preheated sugar solution to each preheated char test tube. To remove air, stopper and invert the tube twice before placing the screw cap over the teflon tape tightly.
6. Place the test tubes on the end-to-end shaker (with help of rubber bands) inside the oven at 75° C and rotate the shaker at 14 rpm for desired period of time:
Rate of Adsorption for 1 hour,
Adsorptive Capacity, 4 hours.

7. After decolorization filter each sample through 0.45 μ M.F. membrane in a filtering flask, including the blank.
8. Adjust pH of the decolorized solution to value of 7 pH = .1 with dil HCl or NaOH, including the blank.
9. Read percent transmittance of the solutions at 420 nm in a 1 cm cell in a spectrophotometer.

V. Calculations:

Rate of Adsorption or Adsorptive Capacity =

$$\frac{\% T \text{ of char sample} - \% T \text{ control sugar liquor}}{\% T \text{ new reference char} - \% T \text{ control sugar liquor}} \times 100$$

VI. Reproducibility of the Batch Test

The reproducibility of the batch test ranges from 1 to 4 % of the results depending upon the nature of the char with an average of about $\pm 2\%$.

VII. Reference Material:

Each refinery may select their own reference standards with respect to feed material. However, a common reference raw sugar should be established and maintained for inter- and intra- refinery char surveys for long term comparison.

DISCUSSION

J. C. ABRAM (British Charcoals and Macdonalds): It was very enjoyable to listen to someone who is still taking an interest in one of the best refining aids available, bonechar. One of the problems in determining the activity of small samples of bone char lies in ensuring that the particle size distribution within the sample is representative of the bulk. When it comes to comparing basic activities, it is necessary to eliminate the effect of particle size and I would recommend the use of narrow sieve fractions, for example that fraction passing through a BSS 18 mesh but retained on a BSS 25 mesh. Another point, you mentioned the use of a standard reference liquor for assessing the various activities of the char. I would suggest that it is easier to keep a reference char than it is to keep a reference liquor.

It is certainly useful for refiners to be able to compare their char stocks with a reference standard. Of greater use is their ability to be able to determine precisely why any particular char stock differs from the standard. Has the total surface area fallen? Are the pores blocked with excess carbon or by inorganic deposits? The tests developed by Bennett and Abram allow one to do precisely this.

C. C. CHOU: We do use a reference liquor and a reference char. That is the only way to compare quality of various chars over a ten year period. For those who are interested in the next paper, try out our procedure on the batch test. You will find that it is very useful.

G. Irvine (British charcoals and Macdonalds): Could I raise several points; 1/ How, from the results of your tests would you be able to assess which properties of the bone char are sub-standard? 2/ I feel that the problem with using liquors is the question of getting a standard which will last you for a long time. 3/ And the other thing is how would you assess if the property which could be at fault is a function of the carbon surface or of the hydroxyapatite surface.

C. C. CHOU: It is a fact that it is very difficult to get a reference liquor and a reference char, but in the absence of a better alternative this is still a good way to do it.

M. A. CLARKE: Do you actually store a reference liquor? Do you freeze it? Or do you keep the reference as dry sugar and make up a solution as needed for each test.

C. C. CHOU: We keep it as dry sugar in a conditioned room with constant temperature and humidity. We have been using the same sugar and char for the last ten years, and we have enough to last another 15 years.

MIXING BONE CHAR WITH GRANULAR CARBON

Frank G. Carpenter

Southern Regional Research Center, USDA

HISTORICAL

Bone char has been used for refining sugar since about 1820. The adsorbent has remained almost the same through the years. The cattle bone starting material is certainly the same, and the heat treatment for manufacture has remained at a temperature of not more than 600°C because above this temperature, the bone structure "sinters" and the adsorptive capacity is rapidly lost. A very small amount of air in the heated zone "activates" the bone char. Improvements through the years have been made in the selection of bone and in control of the manufacturing to produce a more uniform material. The amount of bone char required to refine sugar 80 years ago was 100% char burn/melt. It is only 1/10 of that today, but this is the result of better sugar more than better char. For many years, bone char has been considered the ideal material for refining sugar. So much so, that when E. P. Barrett (Barrett and Brown, 1949) was devising his synthetic bone char, known as "Synthad", he tried to make it as much like real bone char as he possibly could. He was eminently successful in that all of the properties of his Synthad were within a few percent of the properties of real bone char. This had a practical basis, too, because then Synthad could be mixed with natural bone char.

Granular activated carbon was developed by the Pittsburgh Coke and Chemical Co. during World War II as a gas mask charcoal to supplement the short supply of coconut shell charcoal. The very expensive coconut shell charcoal was known to be effective in decolorizing sugar liquor so, after the war, the granular activated carbon was tried on sugar liquors (Gillette, 1956), in spite of the fact that most gas adsorbent carbons do not perform well in solutions. But it does work, and has about 10 times the adsorptive capacity of bone char for sugar colorants. It was made from a particular seam of coal by heating to about 1000°C and steam was used for "activation". It was clearly quite a different material from bone char. In

order to maintain its high capacity for sugar colorants, it was considered to be necessary to regenerate in steam at a temperature near 1000°C. This was clearly above the temperature that bone char could stand, so it was considered that the two could not be mixed. This was 25 years ago and thus it has remained until just recently.

SURFACE CONSIDERATIONS

The carbon content of bone char is about 6 to 8 %, and that of granular carbon is about 60 to 80 %. This fact alone was originally sufficient explanation for the higher capacity of granular carbon. However, only shortly before granular carbon was developed, Brunauer, Emmett, and Teller (1938) had developed their method for evaluating the internal surface of porous adsorbents. The area available to nitrogen molecules for new bone char was 100 sq m/g, and for granular carbon it was 1000 sq m/g. This also explained the 10-fold difference between the two adsorbents.

Then came the realization that sugar colorant molecules are certainly larger than nitrogen molecules and that if the surface area is in very small pores, then that area will be unavailable to the sugar colorant. Measurements revealed that considerable area of both bone char and granular carbon was in very small pores, but that both had substantial area in pores surely large enough for sugar colorant molecules (Barrett, Joyner and Halenda, 1950). This led to the concept that either could lose the area in the small pores but still retain all their adsorptive activity toward sugar colorants. Granular carbon, having more area could lose more and still retain its activity.

In use, the carbon surface is covered with adsorbed organic impurities from the sugar solution. These materials are adsorbed so strongly that they can never be washed off or desorbed. They must be destroyed in place by heat. This slowly leaves a carbonaceous residue that will cover the original surface and block the pores, starting with the smallest. For this reason, an oxidant of air or steam is included in the regeneration. The avowed purpose is to remove this build up of carbonaceous residue, to keep the pores open, and to expose the original active surface. The "original" surface was itself created by pyrolyzing organic material, either when the bone char was manufactured or in the geological past in the formation of the coal, so the original surface is not fundamentally different from the "new" surface covered with recently deposited carbonaceous residue. It is more important that the surface be in an oxidized or oxygen-rich state because oxygen seems to be the key to sugar colorant adsorption.

So, the activity of the carbon surfaces of either bone char or granular carbon are more a function of the use and

regeneration than of the original starting material. If they are mixed together and used and regenerated identically, their surfaces should become alike.

COLOR REMOVAL

Bone char has a significant inorganic phase; the bone mineral which has a calcium rich surface. Granular carbon has no such second phase. This second phase in bone char has a triple effect; it acts as an ion exchanger, as an additional adsorbent, and there is a significant interaction with the carbon surface. Although both granular carbon and bone char certainly must adsorb many easily adsorbed organic molecules equally well through their very similar carbon surfaces, a molecule with little adsorption potential on carbon but with a little ionic nature, even very weak, will be better attracted to the double mixed adsorbent that is bone char. This makes bone char and granular carbon adsorb at least some different colorants. This is also indicated by the fact that although granular carbon may adsorb more total colorant, the first runnings off granular carbon are never quite as colorless as the first runnings off bone char. The double nature of bone char makes it more effective against the very lowest levels of small colorants.

ASH REMOVAL

The ion-exchange properties of bone char combined with adsorption on the bone mineral always results in considerable ash removal by bone char. The monovalent ions such as potassium and chloride are not removed at all, but the polyvalent ions are quite well removed. The balance of colorant adsorption and ash removal in bone char has always been that the colorant removal function was exhausted before the ash removal function was used up. Granular carbon has no ash removal power (in fact it is a little negative), so a mixture of the two can bring the balance out to just even, a very fortunate state of affairs.

pH CONTROL

The ion-exchange function of bone char doubles as a pH control, which works very well. In granular carbon there is no pH control so one has to be added. Advantage is taken of the very slow hydration (literally years) of magnesia. Dead burned magnesite has been found to hydrate and thus supply OH ions at just about the same rate that sugar liquors produce H ions when in contact with carbon. This very small solution of magnesium represents the negative ash removal of granular carbon. A mixture of the two should not require the magnesite.

REGENERATION

The big question is whether bone char and granular carbon can be regenerated while mixed together. A temperature high enough to "properly" regenerate granular carbon would be too high for bone char and would cause it to "sinter" and be "overburned". A temperature right for bone char would be too low for granular carbon and would plug the pores and not completely regenerate it. Recent work by Abram and Bennett (1976) has shown that contrary to previous investigations, bone char can stand a much higher temperature. The older work by Barrett (1962) was concerned with new bone char. Perhaps service char with its multitude of impurities picked up from the sugar is more resistant to high temperatures. It has also recently been shown (Abram and Bennett, 1978) that "overburned" bone char is not caused by the reduction of sulfate by carbon to form sulfide, but rather by the roasting of calcium salts to calcium oxide at high temperatures. "Overburned" char can be made suitable for use by a water wash. Other recent work by Reed (1979) has shown that granular carbon can be successfully regenerated at a lower temperature by using a much longer heating time. Many refiners have for years successfully regenerated granular carbon at temperatures which the manufacturers considered too low. This results in some pore blocking but the smallest pores are not useful anyway. For a mixture of the two, it would appear that the best compromise conditions would be a temperature as high as possible without unduly overburning the bone char, and then a sufficient time to regenerate the granular carbon but allowing some pore blockage.

HARDNESS

By hardness is meant the resistance to mechanical wear. This factor has been emphasized, probably beyond its due. If the regeneration were always perfect and the adsorbent were always returned to its new condition on every cycle of regeneration, then hardness would be important because the char could last forever, except for mechanical wear. But in reality, regeneration is never perfect and there is always some build up of adsorbed impurities that are not removed by regeneration. These materials have to go some place and are only removed when the adsorbent is thrown away. By this reasoning, bone char is too hard. Every bone char system builds up a content of very dense hard inactive and useless particles. These must be removed by a specific gravity separator. If the char were softer these inactive particles would wear away to dust and their removal would be accomplished automatically by dust removal. Granular carbon, when used by itself, does not have this problem, partly because it is soft enough to wear away at about the right rate and partly because it is burned away in the regeneration. In the mixture of the two, the granular carbon should be softer than the bone because the granular carbon part will be the most under-regenerated.

New adsorbent mixture added to the system should be higher in granular carbon than is desired in the operating service adsorbent because it will wear away faster.

SUMMARY

The various properties of bone char and granular carbon have been considered with regard to mixing the two adsorbents. The system is patented (Reilly, 1981) and is now in operation at several sugar refineries so there is no doubt that it is workable. Details of the new operations are described by Barton and Knebel (1982) and by Sulick (1982).

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DISCUSSION

W. R. TUSON (Colonial): I enjoyed your paper and particularly that you reminded us of some fundamentals which we tend to forget. Apparently there is reasonable compatibility between the new granular carbons and bone char regarding the temperature of regeneration. However, there is somewhat different requirements in the oxygen levels. Suppose that in order to minimize the loss of the granular carbon component (which is the expensive one) you have to drop the oxygen level of the regeneration environment. Under such a circumstance it would seem to me that there would be a tendency to increase the inactive carbonaceous residue in the bone char phase which is 80% of the adsorbent. Such a trend over a period of time could very well markedly affect the efficiency of 80% of the adsorbent. You would ultimately reach a point where you had lost considerable ground in the overall capacity of the whole system.

F. G. CARPENTER: The required oxygen level depends upon the individual system and the load of organic impurities that have been adsorbed by the char. Those who have more than one char system know that char with a heavier loading requires a higher oxygen concentration to burn off the heavier loading. You have pointed out a weak spot in the system of mixed adsorbents; how do you keep the balance of carbonaceous residue on both adsorbents? Or even more important; how do you measure the balance of carbonaceous residue on both members of the mixture? There is even a question of how do you measure the amount of granular carbon and bone char in the mixed adsorbent after hundreds of cycles? I suspect that in the long run you will use enough oxygen to properly regenerate the bone char, and this will burn away too much granular carbon. This is the price that you will have to pay for doubling the capacity of the char system.

MIKE FOWLER (Amstar): What happens to the heavy metals that bone char removes from the sugar?

F. G. CARPENTER: The heavy metals accumulate in the hydroxyapatite phase of the bone char. They are very insoluble and are discarded with the discard char.

S. E. GEORGE (B. C. Sugars): Those who stay for SIT will find out that we are using the mixed adsorbent system. Would you speculate on how long it will take to tell how the system is working? We all know that a char house is a very slowly changing thing.

F. G. CARPENTER: I will emphasize that you are right, that a char house is a slowly moving thing. It has a large inertia; a tremendous reserve capacity. But, sooner or later, you can ruin your char. Char can be mistreated for a long time, but not forever. I will take a guess that char can only stand 6 months (or 25 cycles) of mistreatment. So, you should know whether the new system is working in the long run in this time frame.

C. J. NOVOTNY (Industrial Filters): In the mixed adsorbent system you said that regeneration should be carried out so that the bone char was only slightly overburned. I don't think that there is any such thing as slightly overburned. It is either overburned or it isn't. It is like being slightly pregnant. I believe that this will cause something to happen, and I agree with you that it will happen in less than one year.

C. C. CHOU: One of the questions that bothers me the most about the mixed adsorbents is that if you are able to regenerate the granular carbon at low temperature, why then in the water treatment industry, where most of the granular carbon is used, are they not regenerating the carbon at a lower temperature to save energy?

F. G. CARPENTER: They do not use a lower regeneration temperature in the water industry because the water industry is not refining sugar. They adsorb different things out of water than we do out of sugar. Also they may have been oversold by the zealous carbon manufacturers who want their product to be regenerated to new condition so that it will continue to perform always like virgin carbon. This may not be necessary.

C. C. CHOU: As a comment, I feel that there is no question that you can mix the two adsorbents. It is a matter of simple economics.

E. D. Gillette (Refined Sugars): Many years ago we did regenerate granular carbon in a Herreschoff furnace built for bone char. At the time we did it we arbitrarily doubled our retention time on the theory that reactivation was a function of time and temperature. Were we wrong in doubling our retention time? If we were, then if we double retention time

in our char furnace now, we will have lost 50% of our regenerating capacity to gain the extra adsorbent capability.

F. G. CARPENTER: The temperature is in the exponent. The time is just a factor. But, it is a changing temperature, so it is the integrated time at temperature that really counts. The doubling of retention time was a good first guess. Yes, you must lose regenerating capacity to gain adsorptive capacity, but now you do not need to regenerate so much adsorbent. These two just about balance out.

Proc. 1982 Conf. on Sugar Processing Research pp. 47-72

PHENOLICS IN SUGAR PRODUCTS: THEIR ROLE IN FLAVOR AND COLOR PRODUCTION

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INTRODUCTION

Phenolic compounds are a fascinating and heterogeneous group of compounds found in higher plants in a bewildering array of forms. They are responsible for much of the pigmentation in leaves and flowers, contribute to disease resistance, and participate as structural elements in the form of polymeric lignins. In addition, they are responsible for many different types of commonly recognized spice and herb flavors, and are extremely useful in taxonomic studies. They were among the first group of plant products studied. In fact, the first formally organized society of plant chemists, the Plant Phenolics Group, was established to study their chemistry (Swain et al. 1979). Interest in plant phenolics of all types continues to this day.

Studies on the phenolics in sugarcane and sugarbeet are primarily prompted by their ability to enter into color-forming reactions. This was recognized as early as 1916 by Schneller, who evaluated the dark colors formed in cane juices. He concluded that this was due to the reaction of polyphenols with ferric salts and suggested ways to eliminate the problem.

Farber and Carpenter (1975) reviewed the subject of phenolics in sugarcane up to 1972. They identified 21 phenolic constituents; of these, ferulic acid and p-coumaric acid persisted into refined sugar.

Australian workers have extended the investigation of phenolics into new separation techniques, including two-dimensional thin layer chromatography (Paton 1978), prefractionation with columns of XAD-2 resin, Sephadex LH-20, and polyamide (Linecar et al. 1979), and high performance liquid

chromatography (Curtin and Paton 1981). Additional phenolic compounds have been identified by these means.

The fate of phenolic compounds during processing has been investigated as well. Indian workers (Sharma et al. 1978, 1979, 1980) found that carbonatation removed phenols more efficiently from raw juice than did either defecation or phosphatation. They found that phenolic content was higher in young tissue and suggested that its use be avoided. Although carbonatation was the most effective method, it only removed 34% of the total. Increasing dosages of milk of lime increased removal slightly. Additional P_2O_5 could remove up to 45% of phenolics but with detrimental effects on the process. Overall, about 60% of total phenolic compounds inevitably passed through the process of clarification. There was a correlation of 0.705 between phenolic content and color in plantation whites. Sulphitation was found to remove the least amount of phenolics.

Gupta and Srivatsa (1980) concluded that carbonatation sugars had a higher phenolic content than did sulphitation sugars, in contrast to the above findings. However, the quantitative differences between the two methods of clarification were slight and more highly correlated to crystal size, indicating greater inclusion of phenolic colorant in larger grains.

Ito et al. (1980) isolated a fraction responsible for foaming in sugars, which was felt to be closely related to saponin, a phenolic steroid.

In an investigation of constituents that affect settling in cane juice, Sharma (1978) found that juices to which tannins were added refused to settle at all, a situation occasionally encountered under Indian conditions. Other added constituents such as starch, phosphate, silica and nitrogen did not have an adverse effect on settling.

Godshall (1975) followed p-hydroxybenzoic acid through the refining process and found that 50% was removed by phosphate clarification and that only freshly regenerated char or resin could remove this constituent; partially spent char contributed to the load.

In recent years, a great deal of emphasis has been placed on the role of phenolics in the formation of enzymatic colorant. A series of elegant research has been done on both cane sugar (Gross and Coombs 1976a; Coombs and Baldry 1978; Goodacre and Coombs 1978; Goodacre et al. 1980; Coombs et al. 1980) and beet sugar (Gross and Coombs 1976a, 1976b; Madsen et al. 1978; Winstrom-Olsen et al. 1977, 1979; Winstrom-Olsen 1981; Nielsen et al. 1980) in which color produced by the browning and polymerization of phenols by phenol oxidases and tyrosinases was investigated. It was concluded that more

than half of total juice color could be attributed to enzyme reactions (Goodacre and Coombs 1978). Attempts to inactivate the enzyme in processing (Coombs et al. 1980) by various chemical inhibitors and steaming were not practical. The use of heat treatment produced a strong off-flavor in the sugar. The color caused by polyphenols in beet and cane juices was likewise considered to be a main factor in forming color substances that entered the sugar crystal (Madsen et al. 1978). Color formed by polyphenols was of a higher molecular weight than that formed by the reaction of amino acids with sugars. The amino-phenol compound, 3,4-dihydroxy-phenylalanine (DOPA) was the most reactive color former in beet (Madsen et al. 1978; Nielson et al. 1980), while chlorogenic and caffeic acids performed this function in cane (Coombs and Baldry 1978; Coombs et al. 1980).

Color-Forming Reactions of Phenols

Phenolic compounds are very reactive and can participate in several different color forming reactions, all of which are relevant to sugar processing conditions (Singleton 1972). These are briefly summarized here.

Oxidized brown pigments. These are produced both by enzymatic action as detailed above and by non-enzymatic means. The mechanism is the same, involving free-radical formation and random polymerization leading to high molecular weight brown products.

Phenol-aldehyde condensation. This reaction is the basis of several colorimetric tests involving phenols such as vanillin in HCl to determine catechin, and the resorcinol test for carbohydrates. The mechanism involves a condensation between resorcinol and the acid decomposition products of sucrose (HMF) to give a red color.

Phenol-amine reactions. Intensely colored red-brown and bright red products are formed from the reaction of quinones with amines. These are also condensation reactions which ultimately lead to polymeric brown or black pigments known as melanins.

Reactions with metal ions. The most reactive ions toward phenolics are ferric and ferrous salts, which produce an array of pigments with many phenolic compounds, especially those containing o-dihydroxy groups.

Enhancement of other reactions. Reactions between phenols and reactive aldehydes such as furfural and HMF during caramelization (i.e., thermal degradation of sucrose) enhance and speed browning due to concurrent phenol-aldehyde reactions. In addition, quinones, which are oxidized phenols, participate in carbonylamine reactions and can enhance the

maillard sugar-amino acid browning reactions. In all cases, the presence of metal salts, especially iron, further enhances color formation.

Contribution to Flavor

In addition to their contribution as color-producing constituents, phenolics play an important role in flavor and color production in many foods. Their contribution to the flavor of sugar products is not well characterized. Godshall et al. (1979) compiled a list of constituents known to contribute to flavor in sugar products. The list contained several phenolic and other aromatic compounds. In addition to contributing desirable flavors such as that contributed by vanillin and maltol, many of the simple nonvolatile phenolic acids identified in sugar products have bitter and astringent flavors (Maga 1978). They also exhibit a strong synergistic effect on lowering the flavor threshold when used in combination (Maga and Lorenz 1973) with each other.

Methods of Analysis

Numerous methods exist for the analysis of phenolic compounds which are related to their chemical and structural characteristics. Some of the more commonly used methods are listed in Table 1. As with all methods that analyze a group of related compounds, there are interferences and disadvantages associated with each method. A few of these are listed in Table 1. This does not, however, necessarily preclude their usefulness for various applications. In this study, we have evaluated adsorption to insoluble polymers, and detection by UV adsorption, ferric chloride, and Folin-Ciocalteu reagent.

During the course of our work on isolating and identifying compounds responsible for flavor in sugar products, we decided to investigate if it was possible to selectively remove these constituents from the bulk of the sucrose. Selective adsorption of phenols by adsorbents has been used to remove and concentrate them from solution. Among the most commonly used are polyvinylpyrrolidone (PVPP) (Anderson and Sowers 1968; Cornwall and Wrolstead 1981), Sephadex LH-20 (Delcour et al. 1981; Strumeyer and Malin 1975), alumina (Winstrom-Olsen et al. 1977), polyamide (Duggan 1969), and the macroporous nonionic resins XAD-2 and XAD-4 (Linecar et al. 1979; Cornwall and Wrolstead 1981). XAD-2 resin has been used by several investigators to remove sugar colorant (Linecar et al. 1979; Smith 1978; Chou and Rizzuto 1975; Parker and Williams 1969) for further fractionation by other means.

Table 1.--Common Methods for the Analysis of Phenolic Compounds

Method of Analysis	Applicability to Sugar
Prussian blue method (ferrocyanide, ferric chloride) Budini et al. 1980	Reducing substances interfere.
Folin Ciocalteu	Reducing substances interfere. Wide range of reactivity with many compounds.
Adsorption to insoluble polymers	Different adsorptivities; some adsorbents better than others; some irreversible adsorption.
Ferric choride, Rochelle salt, to develop violet color (Kaluza et al. 1980)	Depends on steric configuration of molecules; sugar colorant does not react.
Vanillin-HCl method (Maxon and Rooney 1972)	Specific for catechins and leucoanthocyanins. Sugar colorant does not react.
Enzymatic (polyphenol oxidase) (Worthington 1978, Nelson and Mason 1976)	A new method in sugar industry; some substrate specificity; sugar may interfere at high concentrations; best for juice analysis.
UV absorption	Simple and very popular. Interference from proteins, nucleic acids, sugar colorant. Wavelength specificity.
Aminoantipyrine method (Toras et al. 1965)	Yellow complex formed is similar to sugar color; requires distillation and thus best for volatile phenolics. Approved EPA method.
Ferric chloride	Many different colors; yellow complex formed with sugar constituents similar to reagent color. Methanol inhibits complex formation.

EXPERIMENTAL

Purification of adsorbents. The XAD resins were washed first with methanol under filtration until no color or turbidity eluted. The procedure was repeated with water, 1% NaOH, water to neutrality, 10% HCl, and water to neutrality. Resins were stored immediately after filtration so that they were still hydrated but not wet. Alternately, they were stored in methanol for longer period of time, but needed to be rehydrated prior to use.

Alumina was boiled about 5 min in 5% HCl, washed with water until neutral, and oven dried.

Polyvinylpyrrolidone (cross-lined) was purified according to the method of Anderson and Sowers (1968) which consisted of acid-washing and testing for reducing substances with ferrocyanide. In addition, it was washed and filtered several times with acetone and air dried.

bone char (new) and ion exchange resin (new) were supplied by sponsoring companies and used as received.

Comparison of adsorbents. To compare the ability of the adsorbents to remove compounds that gave a phenol reaction, 5 ml of 1% solution of a soft sugar was treated with weighed amounts of adsorbents in 15 ml serum vials, stoppered, and shaken on a wrist action shaker for 30 min. Three pH levels were investigated. The pH was adjusted to 3.5 with acetic acid and to pH 9 with 1% NaOH. pH unadjusted samples were also tested. Adsorbent was removed by centrifugation and the phenol value, after treatment, was compared to that prior to treatment. The weight of adsorbents used per 5 ml were the following:

XAD-4, 0.9 g
XAD-2, 0.9 g
Sephadex LH-20, 0.9 g
Alumina, 0.5 g
PVPP, 0.2 g
Bone char, 1.0 g
Ion exchange resin, 0.8 g

In addition, solutions of known phenolic and other phenolic-reacting compounds were reacted in the same manner. Solutions of approximately 0.1 mg/ml (100 ppm) were used. When necessary, solution were diluted 1:10 to determine their absorptivity (a) so that the percentage removed by each adsorbent could be calculated. Absorptivity was calculated in the following manner:

$$a = \frac{A_{650}}{\text{conc., mg/ml}}$$

Analysis of phenolic substances. Several methods were evaluated for the analysis of phenolic substances. Absorbance at 320 and 330 nm was taken. In addition, solutions were reacted with ferric chloride and with Folin and Ciocalteu Phenol Reagent (Sigma).

The ferric chloride test was the following: To 2 ml of aqueous sugar solution (10-20%) was added 0.2 ml ferric chloride reagent, which consisted of 2% ferric chloride, 25% methanol, and 1% HCl in aqueous solution. Methanol was required to prevent turbidity of the reaction mixture and HCl was required to prevent oxidation of the ferric chloride to the oxide. However, an excess of methanol inhibited the reaction. The absorbance of the complex was read at 480 nm, against a blank consisting of sugar solution and 0.2 ml water. In addition, the absorbance of a solution containing 2 ml water and 0.2 ml ferric chloride reagent was subtracted from all absorbance readings. This resulted in linearity upon serial dilution. The ferric chloride units of each sugar were determined using the same calculation as for phenol units, detailed below.

The phenol test was the following: To 2 ml of aqueous sugar solution (1-2%) was added 0.2 ml phenol reagent and 0.4 ml 2N NaOH. The reaction mixture was thoroughly stirred for about 5 min and the absorbance read at 650 nm against a blank containing all reagents plus water instead of sample. Phenol units were calculated as follows:

$$\text{Phenol Units} = \frac{\text{Abs } 650 \times 1000}{\text{mg/ml sugar}}$$

Extraction of soft sugars. A 3 cm x 40 cm column was filled to a depth of 33 cm with XAD-2 or XAD-4. A 30 Brix solution containing 100 g soft sugar, adjusted to pH 3.5 with acetic acid was eluted through the column at a rate of 6 ml/min. The column was washed with 2 liters of water to remove the sugar, and the adsorbed material was desorbed with 500 ml methanol and 200 ml 50% acetone. The eluates were combined and evaporated to the water phase under vacuum and lyophilized. In this manner, a coating syrup, a light brown sugar, a dark brown sugar, and a turbinado sugar were extracted by XAD-2. The light brown sugar was also extracted by XAD-4. The resulting solid material was extracted with chloroform and analyzed by gas chromatography. Regeneration of the column was similar to the purification procedure outlined above for the resins, namely, successive washes with water, NaOH, water, HCl, and water until neutral.

Gas chromatography of extracts. The chloroform extracts of the XAD material were evaporated to dryness in 2 ml vials and dissolved in a minimum amount of pyridine. The silyl

derivatives were made with a 2:1 mixture of hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS).

Chromatography was conducted on a Model 5880 Hewlett Packard Gas Chromatography utilizing a 12 meter fused silica capillary column. Helium was used as the carrier gas, at 1 ml/min, with 30 ml/min make-up gas. The split ratio was 1:22. The program used for most of the separations was the following: 130°C for 2 min, increase temperature 4°C/min to 280°C and hold for 6 min.

Mass spectrometry was conducted on a Finnigan 2000 as described elsewhere (Godshall et al. 1979).

Evaluation of the flavor of XAD-2 extract. A large column was packed with XAD-2 resin to dimensions of 7.5 cm x 26 cm. The resin was extensively purified on column as described above. 400 g of a high quality light brown sugar was dissolved in 1200 ml water and the pH adjusted to 3.5. This solution was eluted through the column and the sugars washed out with about 10 liters of water. Material was desorbed with 750 ml methanol followed by 800 ml 50% acetone. The column was again washed with 4 liters of water and the reserved, once-adsorbed solution was re-eluted and the procedure repeated to remove adsorbed material. The solvent fractions were combined, evaporated down to a small volume under low pressure and temperature and lyophilized. This resulted in 700 mg of solid material.

A fondant was made from 400 g Sanding sugar, utilizing the XAD-2 elute as the only source of color and flavor by the method of Christenson and Anhauser (1980). The resulting candy was informally evaluated by 7 people who were familiar with the flavor of brown sugars and who had been training to recognize various flavors.

RESULTS AND DISCUSSION

The Folin-Ciocalteu phenol test is based on the ability of aromatic hydroxyls to be oxidized by and to form a complex with the phosphomolybdate reagent, which turns blue on addition of alkali. Since it reacts to a greater or lesser degree with all reducing agents, we decided to test a number of compounds known to be in soft sugar products for their reaction with the reagent. The results of this survey are listed in Table 2. The most striking aspect of the results is that, while very many nonphenolic compounds reacted with the reagent and thus were a source of severe interference, it appeared to react with most of the flavor-causing substances in soft sugars, without serious interference from invert, ash, or acetic acid, a major volatile in these products. This aspect may thus hold some promise in flavor evaluation of soft sugars.

Table 2.--Reaction of Various Constituents with Phenol Reagent

<u>Sugars</u>	<u>Reagent</u>	<u>NaOH</u>	<u>Next Day</u>
Sucrose	None	None	None
Glucose	None	None	None
Fructose	None	Blue	Same
Sorbose	None	Light blue	Same
<u>Sugar Products</u>			
ISP ¹	None	Light blue	Faded
Raw sugar dialyzate	Green	Blue-black	Green
Soft sugars	None	Blue	Same
Caramelized sugar	None	Blue	Same
Caramel #105	None	Black (2000) ²	Same
Caramel #201	None	Black (5000)	Same
<u>Salts</u>			
Na-meta-silicate	None	None	None
ferric chloride	Blue + white ppt	Brown + ppt	Blue
Ferrous sulfate	Blue, darkening	Same	Same
<u>Non-phenolic compounds</u>			
2-furyl-ethyl-ketone	None	Med. blue	Faded
MMF	None	Pale blue	Yellow
Isomaltol	None	Med. blue	Faded
Acetyl formoin	Blue	Black	Same
2-Acetyl furan	None	Faint blue	Faded
Maltol	None	Deep blue(26250)	Same
5-Methylfurfural	None	Deep blue-green	Green
Furaneol	Lt. blue-green	Blue-black(30090)	Same
Acetic acid	None	None	None
Furfural	None	Med. blue	Brown
2-methyl-butanal	None	Blue	Same
Diacetyl	None	Green (8500)	Brown
Furfuryl alcohol	None	Dark blue	Same
Hydroxyacetone	None	Dark blue	Same
Maple lactone	None	Deep blue (30630)	Same
2,3-pentanedione	None	Blue-black	Same
Pantolactone	None	None	None
Butyrolactone	None	None	None
Phenylacetic acid	None	None	None
o-Orn-phenylacetic	None	Blue (42310)	Same
2,3-butanediol	None	None	None
Imidazole	None	Blue-green	Green
Coumarin	None	Dark blue	Same
Benzo-C-furan-2-one	None	None	None

¹Indigenous sugarcane polysaccharide

²Numbers in parenthesis indicate the calculated phenol value, which is comparable to the phenol value of soft sugars. It is calculated as absorptivity (Absorbance/conc in mg/ml) x 1000.

The ferric chloride test detects only those phenolics whose structure is spatially acceptable for complexing with the ferric ion. Since the reaction of ferric chloride with phenols results in many different colors, depending on structure, we decided to see if any of the common phenolics known to be in sugar products gave the same sort of orange to orange brown color seen when ferric chloride is reacted with dilute soft sugar solutions. The results are listed in Table 3. Of the compounds surveyed, three had a similar reaction to that of soft sugars, and standard curves were prepared for all three (4-methylcatechol, 3-methoxycatechol, 4-hydroxybenzoic acid), with the results seen in Figure 1.

Table 3.--Reaction of phenolic compounds with ferric chloride reagent in 10% sucrose solution.

Compound	Abs. Max.	Color	Extinction Coeff. (A_{480})
4-Methylcatechol	UV	Orange	154.2
3-Methoxycatechol	400	Orange	729.2
4-Hydroxybenzoic acid	UV	Orange	22.1
Maltol	UV + 500	Violet	
Catechol	UV	Green	
3,4-Di-OH-Benzoic acid	UV + 670	Blue-green	
Caffeic acid	660	Grey-green	
Ferulic acid	--	Grey precipitate	

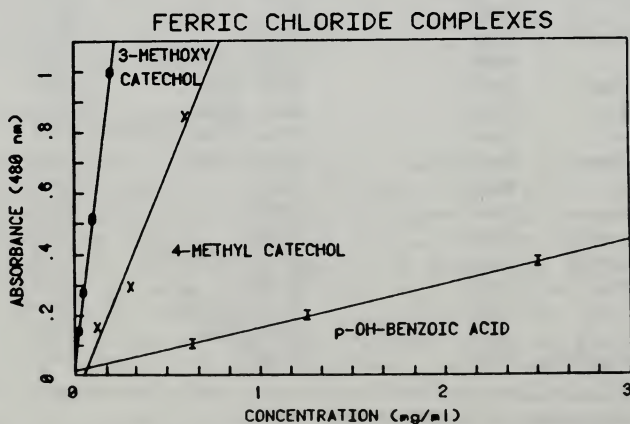


Figure 1. Standard curves for various phenolic compounds with ferric chloride.

Since both these tests showed a great deal of variability in the sensitivity of various compounds to the reagents, it was not feasible or meaningful to choose any one compound as a reference. For this reason, we chose to use the phenol and ferric chloride values which would make it possible to compare one sugar to another. Table 4 lists these values for sixteen sugars. The phenol test is about 20 times more sensitive than the ferric chloride test, and although the ferric chloride method is specific for phenols, the correlation of the two values was 0.90 (Figure 2), indicating that both methods were measuring similar constituents. Because of its superior sensitivity, the phenol test was used to evaluate the effectiveness of adsorbents in removing phenol-reacting substances from sugars.

Table 4.--The phenol value, ferric chloride value, and ICUMSA color of soft sugars

Sugar	Phenol value	FeCl ₃ value	ICUMSA color
1 (Turbinado)	20.7	0.76	1414
2	30.8	1.72	3042
3	34.8	1.52	2875
4	39.8	1.77	2694
5	44.8	3.11	10924
6	53.4	3.60	4163
7	64.4	3.47	6681
8	69.2	2.09	9095
9	76.1	3.31	4244
10	79.8	4.16	16349
11	87.9	3.45	4695
12	91.3	3.59	6989
13	89.7	4.81	15854
14	112.3	4.14	7040
15	115.3	5.14	18916
16	134.2	5.95	13027

Figure 3 shows the correlation of color with phenol value, which was 0.6513. This is in close agreement with the correlation of 0.705 found by Gupta and Srivatsa (1980). Figure 4 shows that the correlation of color with the ferric chloride value is slightly higher, 0.7639. The correlation of ferric chloride value to color suggests that much of the color has phenolic moieties incorporated into it, since this reagent reacts exclusively with phenolics and not with all the other types of compounds present as does the Folin and Ciocalteu reagent.

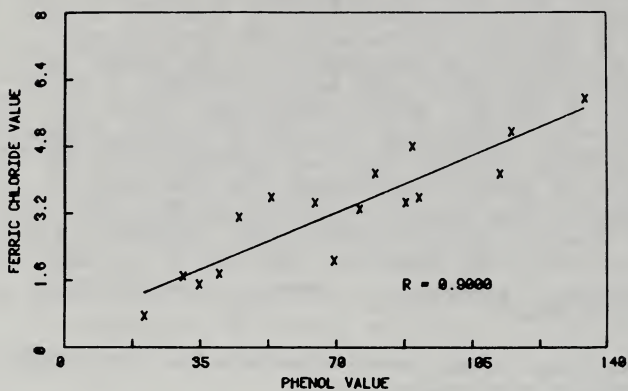


Figure 2. Correlation of the ferric chloride value and the phenol value of several soft sugars.

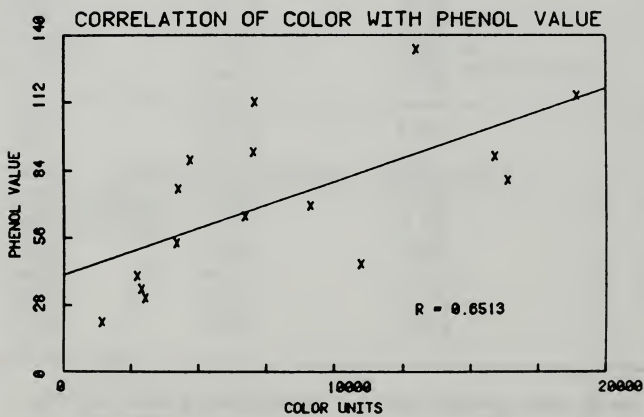


Figure 3. Correlation of soft sugar color with their phenol values.

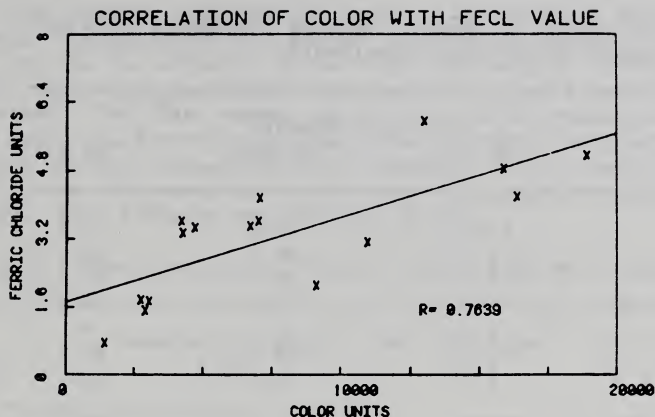


Figure 4. Correlation of soft sugar colors with their ferric chloride values.

The ability of various adsorbents to remove phenol-reacting substances from soft sugar solutions at various pH values is listed in Table 5. The results show that bone char is the most efficient at all pH values. However, the adsorption of compounds to bone char is irreversible. XAD-4 resin at pH 3.5 was able to remove 74.4%. Addition of extra resin did not enhance extraction. Although PVPP is widely recognized for its ability to selectively adsorb phenolic compounds, it was not able to remove any substances from sugar solutions. In fact, in all cases, there was a 19 to 26% increase in color after treatment with PVPP. Blank runs with PVPP in water acidified to pH 3.5 with acetic acid showed that there was a minimal amount of color contributed by the PVPP, but this could not account for such increases. Some preliminary work using ultraviolet detection instead of the colorimetric method showed that certain compounds such as maltol and other non-phenolics caused an increase in absorption after treatment with PVPP, suggesting that a reaction may be taking place between the resin and these types of substances. The ability of XAD-4, XAD-2, and PVPP to adsorb certain constituents out of solution is listed in Table 6. It is interesting to note that the substances PVPP is able to remove all contain a para-hydroxy benzene grouping.

Table 5.--The removal of phenol-reacting substances from soft sugars by different adsorbents

Adsorbent	% Removed		
	pH 3.5	pH unadj	pH 9.0
XAD-4	74.4	67.0	70.0
XAD-2	63.5	40.7	46.5
Alumina	41.7	35.7	37.7
Sephadex LH-20	0	17.1	28.9
PVPP	0	0	0
Bone char	83.3	89.7	90.8
Ion exchange	25.4	32.5	42.7

Table 6.--Comparison of the efficiency of extraction of constituents from solution by adsorbents

Constituent	% Removed		PVPP
	XAD-4	XAD-2	
Maltol	94.4	79.4	0
Catechol	93.4	82.8	51.8
Tannic acid	97.0	93.0	97.0
Diacetyl	92.8	92.1	0
Maple Lactone	94.4	79.4	0
p-Hydroxybenzoic acid	90.8	77.0	79.6
o-Hydroxyphenylacetic acid	78.5	70.7	0

XAD-4 resin was by far the most efficient in removing all types of compounds. However, we found that the resin was difficult to regenerate and it appeared to remove much less of the color from a sugar solution than did XAD-2. We found that, although XAD-2 removed 63.5% of phenol-reacting substances from a solution, it was possible to recover 74.3% of the total phenol-reactivity from the material adsorbed to a column filled with XAD-2. The ease with which XAD-2 resin columns could be regenerated and the fact that so much of the adsorbed material could be recovered led us to choose this resin over the XAD-4 for gas chromatographic evaluation of sugar extracts and for flavor evaluation.

The material desorbed from the XAD-2 column was a dark brown, fine powder. It possessed a sweet caramel or burnt-sugar type of odor reminiscent of soft sugars. However, the flavor was extremely bitter. Table 7 lists some of the characteristics

of this material from several sugars. The extracts were heavily contaminated with sucrose in spite of extensive washing and the low affinity of the resin for sugars. In addition, high molecular weight colorant was included in this fraction. For this reason, the solid material recovered from the resin was further extracted with chloroform to eliminate sugars and high molecular weight material. This resulted in a fraction that amounted to less than 100 ppm of the original sugar. This fraction was analyzed by GLC.

Table 7.--Characteristics of XAD-2 extracts of soft sugars

Sugar	Adsorbed by resin (ppm)	Amt. CHCl ₃ ext. (ppm)	% Sucrose by GLC
Turbinado	654	30	39.0
Light brown	1784	Not determined	32.8
Dark brown	3000	84.5	22.4

GLC results. Figure 5 shows the resulting gas chromatogram of the chloroform extract of the XAD-2 eluate of a coating syrup. The fifteen compounds that were identified by mass spectroscopy and retention times are listed in Table 8.

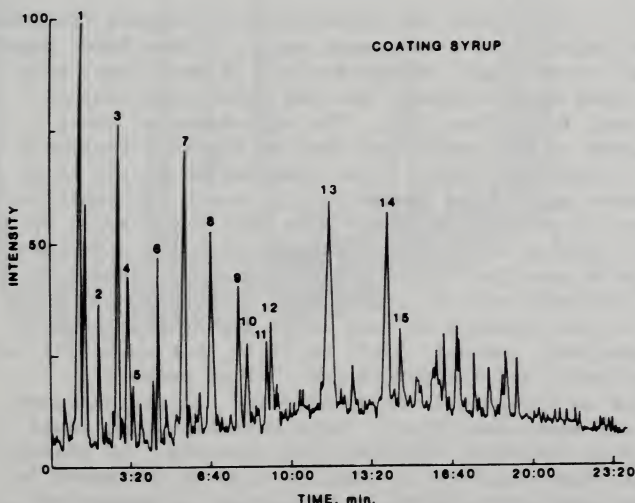


Figure 5. Gas chromatogram of the chloroform extract of the XAD-2 eluate of a soft sugar coating syrup. Numbered peaks are identified in Table 8.

Table 8.--Compounds identified in the chloroform extract of the XAD-2 eluate of a soft sugar coating syrup

Peak No.	Compound
1	Benzoic acid
2	Phenylacetic acid
3	Catechol
4	2-Methyl-benzoic acid
5	Acetyl salicylic acid
6	4-Methyl catechol
7	Acetyl formoin
8	2-Hydroxy-benzoic acid
9	3-Hydroxy-benzoic acid
10	3,4-Dihydroxy-benzoic acid
11	4-Hydroxy-benzoic acid
12	3-Hydroxy-phenylacetic acid
13	Syringic acid
14	4-Hydroxy-cinnamic acid
15	Vanillic acid

This extract was obviously highly enhanced in phenolic compounds. In addition, two highly flavorful and aromatic compounds, phenylacetic acid and acetyl formoin, were found.

Figures 6, 7, and 8 show the chromatograms obtained from the chloroform extracts of a turbinado sugar, a dark brown sugar, and a light brown sugar, respectively. In each case, more than 100 peaks were obtained, but the major constituents numbered much fewer than this. The appearance of these chromatograms is different from that of Figure 5 because a different column was used. Close examination of the three tracing shows many peaks common to all three, with notable differences in relative amounts. The dark brown sugar was noticeably lacking in the large cluster of peaks with retention times between 29.8 to 33.0 min, which was present in both the turbinado and the light brown. Another point of interest is the presence of four large peaks at the end of the chromatogram, present in all extracts, but especially so in the turbinado and the dark brown. These peaks are represented in Figure 9 in enhanced form. These compounds have not yet been identified. Their retention time would coincide with flavonoid aglycones and steroids. At present it has only been possible to identify some of the peaks in the earlier part of the chromatograms, most of which are similar to those found in the coating syrup.

Peaks with retention times between about 21 and 38 minutes are compounds with molecular weights greater than the

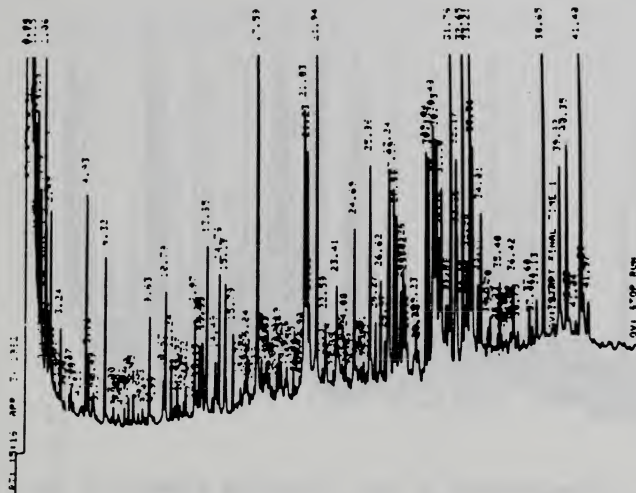


Figure 6. Gas chromatograph of the chloroform extract of the XAD-2 eluate of a turbinado sugar.

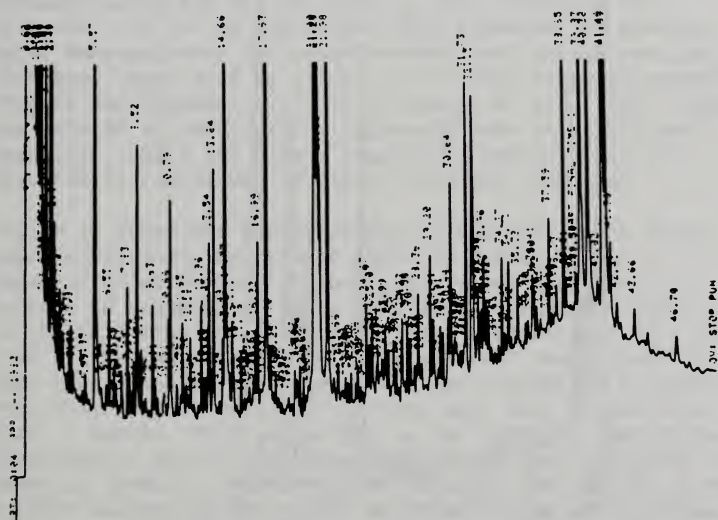


Figure 7. Gas chromatograph of the chloroform extract of the XAD-2 eluate of a dark brown sugar.

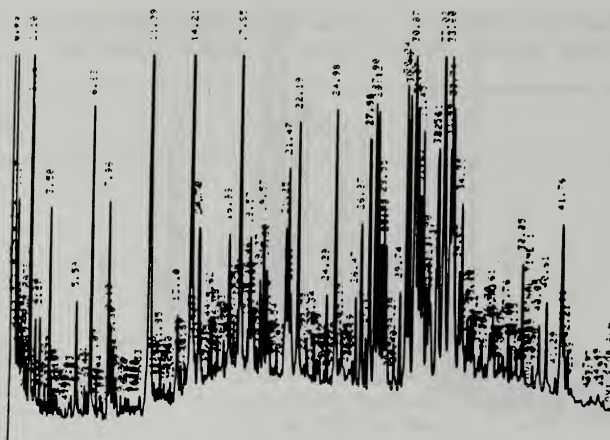


Figure 8. Gas chromatograph of the chloroform extract of the XAD-2 eluate of a light brown sugar.

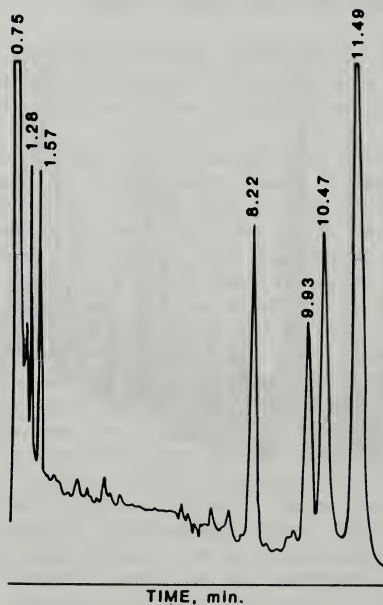


Figure 9. Gas chromatograph of the 4 large unidentified peaks seen in the XAD-2 extract of a dark brown sugar. Program was 270°C for 6 min; 2°C / min to 280°C for 3 min

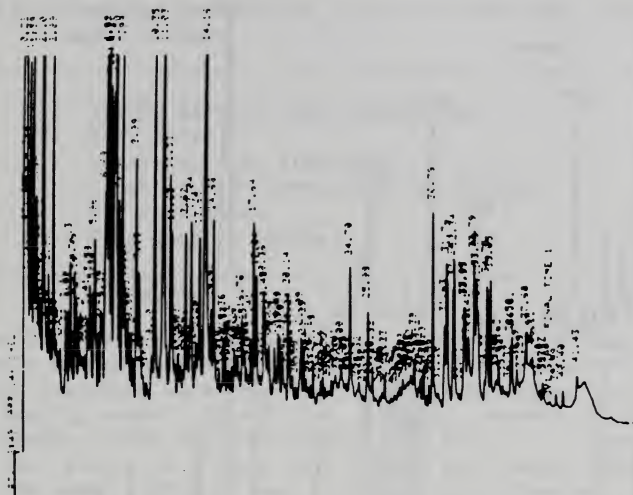


Figure 10. Gas chromatogram of the chloroform extract of the XAD-2 eluate of a light brown sugar.

cinnamic acids but not as large as the flavonoids and steroids. It can be speculated that these may be hydroxylated benzopyranone and benzofuranone derivatives such as have been identified as products of carbohydrate degradation (Popoff and Theander 1972, 1976; Olsson et al. 1979). These are related in structure to compounds such as maltol and isomaltol, which are highly flavorful, and may thus be expected to contribute to flavor in sugar products.

Figure 10 shows the XAD-4 extract of the same light brown sugar whose XAD-2 extract is shown in Figure 8. An examination of the two chromatograms shows that the XAD-4 extract is enhanced in peaks with lower retention times, up to about 15 minutes. Figure 11 shows a comparison of this area of the two extracts by using a faster chart speed to make the peaks easier to compare. The extraction of higher molecular weight compounds was very poor when compared to that of XAD-2. This may be due to the stronger adsorption of compounds by XAD-4, which was shown to be more efficient in removing aromatic compounds from solution than XAD-2. A subsequent elution of the matter adsorbed to the XAD-4 column was made with 1% NaOH, but GLC showed that this fraction did not contain any appreciable number of compounds, indicating that desorption either did not occur, or that these compounds were not adsorbed by the resin in the first place.

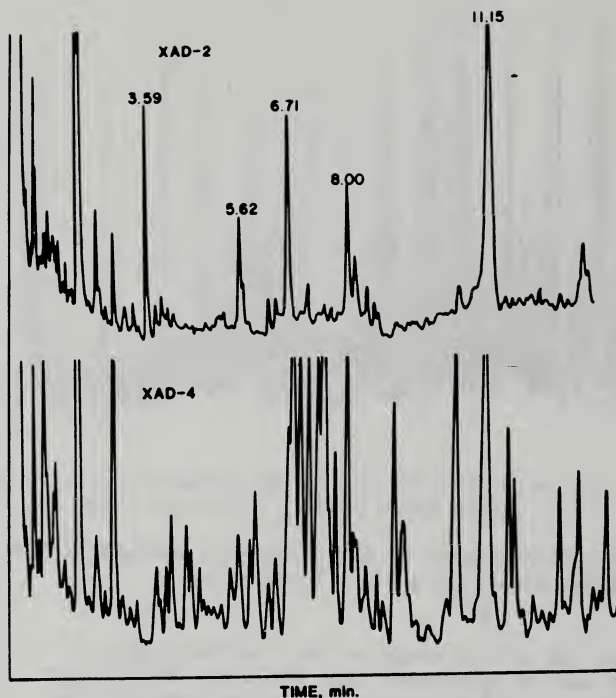


Figure 11. Comparison of the earlier part of the chromatograms of the XAD-2 and the XAD-4 extracts of a light brown sugar.

Evaluation of flavor and XAD-2 extract. The candy that resulted from using the XAD-2 material as the sole source of flavor and color possessed adequate color and flavor so that the tasters felt that it was a brown sugar candy and not one made from refined sugar with only color added. Nevertheless, it did not possess all of the same flavor of the normal soft sugars. This is undoubtedly partly due to the fact that acetic acid was lacking. (We have found that most soft sugars have from 300 to 500 ppm of acetic acid.) Comments received on the flavor are listed in Table 9. Since this was an informal tasting, no flavor scoring was done, but the comments do indicate that much of the flavor and color could be recovered from XAD-2 for further evaluation.

Table 9.--Comments received on flavor of candy made with
XAD-2 extract

-
1. Strong flavor; lacks something
 2. Very salty
 3. Metallic; very flavorful
 4. Slightly off-flavor, a green flavor,
but very tasty
 5. Very good (2 people)
-

The solid material obtained by XAD-2 extraction of sugar products had an extremely bitter taste. Phenolic acids are usually described as bitter, and this may have been the contributing cause. In addition, they may possess slight astringency, which can be recognized as bitter. It appears that when placed in a sugar matrix, the bitterness combines with the sweet flavor to help produce a full-flavored brown sugar. It must be emphasized that the material extracted by chloroform for GLC represented less than 100 ppm of the sugar, and with over 100 compounds present, each one is present in very low quantities. Many are probably at sub-threshold levels. Maga and Lorenz (1973) showed that phenolic acids can synergistically enhance the threshold levels when used in combination with each other.

SUMMARY

In conclusion, we have shown that the nonionic macroporous polystyrene resin, XAD-2, can be used to selectively obtain a fraction from soft sugar that is enriched in the color and flavor of the product. The more highly crosslinked resin of similar composition, XAD-4, is better suited to obtaining a fraction that is enriched in lower molecular weight phenolic compounds. Both resins are able to extract aromatic compounds other than phenolic compounds. The combination of XAD-2 extraction of sugars, followed by chloroform extraction of the resulting eluate, and capillary GLC analysis provides a sensitive method to evaluate the minor constituents of soft sugars and a means of comparing their flavorant profiles.

We have also found that the Folin-Ciocalteu test for phenols reacts with many of the compounds identified in sugar products that contribute to flavor and odor, other than phenolics. The XAD resins are able to extract at least 75% of this reactive material, which could then be recovered for GLC analysis. There is the interesting possibility that the phenol value may be correlated to the flavor of soft sugars. Work is in progress to flavor score all the soft sugars whose phenol value has been determined.

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DISCUSSION

D. I. WANKLYN (Redpath): This work is of extreme importance to the refiner who is making soft sugars, and I hope that we have your assurance that it is going to continue. The problem that we have is that a great deal of color has to be removed from syrup before these sugars can be boiled. If we do a very good job in decolorizing then we tend to lose flavors that should be retained. I don't know what the answer is, but it seems to me that you are heading in the direction to give us some guidance on how to get the off flavor and color out and retain the flavors that sell the product.

M. A. GODSHALL: Yes, we do intend to continue this research. It is a long term research project that can go into any number of ramifications. I have always felt that color and flavor go along with each other, and one of our goals is to determine what that relationship is.

P. SMITH: Can you see the day when we will extract these things specifically and then add them back into a highly decolorized liquor?

M. A. GODSHALL: That certainly would be foreseeable, but at present it would probably not be economically feasible. The refiners do a good job of making acceptable brown sugars now. Someday there may be the need or the desire to make novelty sugars that would have tailored flavors or even sugars with flavor but no color. If we understand the processes of flavor formation and removal, we can then proceed to produce uniform quality products.

SUGAR, NUTRITION, AND HEALTH

G. Norris Bollenback

The Sugar Association, Inc.

Dr. Bolenback's presentation was based on the following material published as "Sucrose and Health", Chapter 6, in "Food Carbohydrates", Ed. David R. Lineback and George E Inglett, Avi Publishing Co., Inc., Westport, Connecticut, 1982.

INTRODUCTION

Let me emphasize at the onset that the point I wish to make is that the sugar and sugar-user industries have been and are very concerned over attempts to associate sugar(sucrose) with health and nutrition problems. Sugar does not stand alone in this respect, for many products of food industries have been similarly involved. The sugar and sugar-user industries have taken steps to reflect their concerns and I would like to describe some of the activities we have developed to defend against unfounded accusations and to better describe the role of sugar in the diet to the many communities making up our population.

Before plunging into the sugar and health issues and what we have done to deal with these issues, it is best to have a clear understanding of what the term "sugar" means. To most people - carbohydrate chemists, sweetener producers and processors, industrial users, government personnel and the consumer - "sugar" means sucrose, the sugar obtained from both sugar cane and sugar beets. Somewhere along the line - possibly with the publication of the Dietary Goals (U.S. Senate 1977) - many in the media started to use the term "sugar" as a generic one to include all carbohydrate sweeteners from sugar cane, sugar beet, and corn. There has been a not too vigorous promotion of the plural word "sugars" as one generic for carbohydrate sweeteners. Whether this will be adopted in the United States remains to be seen. Meanwhile, "sugars" has been declared the generic term for the labeling of carbohydrate sweeteners in foodstuffs in Canada (Anon. 1981A).

In this presentation, the term "Sugar" refers only to sucrose, the sweetener from sugar cane and sugar beets. When the occasion arises, other carbohydrate sweeteners will be specifically defined.

CONSUMPTION

One of the problems issuing from the misuse of the word sugar is the often innocent reporting in the media on the amount of sugar the people in the United States consume in a given year. It is not unusual to read in newspaper or magazine articles that we, the American people, consume almost 59 kg (130 lb) of sugar per person per year (Anon. 1981B). Such statements not only bring out the misuse of the term sugar but also a misunderstanding of the word "consumption."

According to USDA (1981), which is the primary source for sugar and sweetener statistics, the last year for the per capita consumption of sugar to rise to or above 45 kg (100 lb) was 1973 (46.1 kg or 101.5 lb per capita). Since 1973, the USDA shows a downward trend in sugar usage to the point where in 1980 the figure was 38.9 kg or 85.6 lb per capita. The major reasons for this downward trend appear to be the high prices for sugar, especially in 1974, and the impact of high fructose corn syrups in the industrial market place. The difference between the approximately 39 kg (85 lb) for sugar and the often quoted approximately 59 kg (130 lb) is due to use of corn sweeteners (19 kg or 41 lb) and other caloric sweeteners such as honey and edible syrups (0.6 kg or 1.3 lb). Thus the 59 kg (130 lb) figure refers to use of all caloric sweeteners and not sugar alone.

The word consumption is used rather freely where sugar and other sweeteners are concerned. Actually, the per capita consumption data are calculated from annual delivery figures and the population as of July 1 in a given year. The amount of sugar delivered to various industrial, institutional, and consumer stations is not necessarily the amount of sugar consumed. Not taken into account, for instance, are quantities held in inventory and on shelves, lost mechanically, chemically and enzymatically during processing, and discarded at point of use.

For instance, the baking industry currently takes over one million tons of sugar a year (USDA 1981). Perhaps 60% of that sugar is used in the manufacture of yeast-raised bakery goods and about half that amount is consumed by yeast and never reaches the consumer. The point is that the per capita consumption figures for sugar and other sweeteners available and quoted are actually delivery figures and are an overestimate of actual consumption values.

Are there any data available that reflect the actual consumption of sugar by the American people? Not really. The USDA does offer estimates based on market basket surveys (USDA 1980B) and there are data banks in the United States that contain estimates of amounts of simple and complex carbohydrates in the diet. But the act of collecting information is replete with all the problems encountered in dietary surveys plus the lack of analysis on food products as eaten by the consumer. For example, in a preliminary report on the Nationwide Food Consumption Survey 1977-1978 (USDA 1980A), it was noted that one category of foods surveyed (sugar and sweets) included granulated sugars, jams, jellies, and candies, but sugars and sweeteners that are ingredients of other foods are reported with those foods - sweeteners in soft drinks in the beverage group and sweeteners in presweetened cereals in the grain products group.

Analytical data for sweetener composition of consumer food products are scarce. A noteworthy contribution to the accumulation of such data is that of the group under Dr. Kent Stewart of the Nutrient Composition Laboratory of the Nutrition Institute of the USDA. Dr. Stewart (1979) and Drs. Li and Schuhmann (1980, 1981) of Dr. Stewart's lab, have published analyses for simple sweeteners in soft drinks, dried cereals and granola-type breakfast foods. More data of this type are needed in order to give us a better grip on how much sugar is actually consumed in the country. One would wish that Dr. Stewart's analytical work would proceed in a logical, orderly manner rather than be determined, apparently in part, by political motivation.

Even with such data on hand we can wonder whether the inverted sugar found in acidic soft drinks can be considered as sugar consumed. If so, then using the term "sugar" as a generic to include invert sugar (and high fructose corn syrup) might be preferable.

On the other hand, if the body sees sucrose and an equimolar mixture of glucose and fructose differently, then perhaps the better part of the more than two million tons of sugar delivered to the soft drink industry per year (USDA 1981) should not be considered as sugar. The more we know, the more guidelines we need. And it is completely simplistic to claim that a given quantity of a given sweetener is consumed, that that amount is too much, or not enough or just right. We need a lot more information and definite baselines before such claims can become valid.

I have mentioned millions of tons of sugar delivered per year to the soft drink industry. To give us a feeling for the size of the sugar industry let me point out that for the past several years the total sugar delivered to various customers has amounted to ten million tons per year (USDA 1981). That is

20 billion pounds - a rather large amount of any food product. To emphasize the immense volume, worldwide production of sugar from both sugar cane and sugar beet is estimated for 1980-1981 as 86.5 million metric tons (94.9 short T) while world consumption is estimated at somewhere between 89 (98.1 short T) and 91 million metric tons (Licht 1981). That is a lot of sugar.

With that amount of product available and wanted throughout the world there must be something attractive about it, even if it is only sweetness and price or a combination of the two. And, perhaps, with such ubiquity some people are bound to suspect and find iniquity.

HEALTH

In looking at attempts to associate sugar with health and nutrition problems, one of the steps we advise taking is to examine reasons behind the problems.

Some outstanding bookmarks can be found over the past few decades wherein sugar has been associated with various health and nutrition problems. The book by Abrahamson and Pezet (1951) "Body, Mind and Sugar" deals in great part with the experiences of Seale Harris and Dr. Abrahamson in the area of hyperinsulinism (hypoglycemia) and the role of sugar therein. The book seems rather more popular today - it is available in paperback on many newstands - than when it was published. Also worthy of note as a primer for anti-sugar buffs is the publication by Cleave and Campbell (1966) called "Diabetes, Coronary Thrombosis and the Saccharine Disease," in which refined carbohydrates (especially starch and sugar) are singled out as promoters not only of the title diseases but of other gastrointestinal problems.

Perhaps the most often quoted anti-sugar person is John Yudkin (1972) who, in his book, "Sweet and Dangerous" suggests sugar as a cause of heart disease, diabetes and "other killers."

But at the time of publication of these books, the public did not seem to clamor for a closer look at sugar's role in the diet and sugar's possible role in health and nutrition problems. This occurred later in the 1970's. We may possibly assign as catalyst to the eventual public interest, comments made by Bob Choate before a Congressional Committee on Nutritional Content and Advertising for Dry Breakfast Cereals in 1972 (U.S. Senate, 1972). Choate called attention to the involvement of sugar in dental caries and "...the growing medical debate on sugar and its role in our diet, its possible contribution to heart problems and diabetic onsets." References for information cited by Choate were Yudkin and Dr. Jean Mayer.

Dr. Mayer (1976) by-lined an astonishing lay article in the New York Times of June 20, 1976, titled "The bitter Truth about Sugar" in which he incriminated sugar in everything from robbing the body of vitamins to promoting the death of the islets of Langerhans. The article was noteworthy in its subjunctive mood - denoting points conceptually and not backing them by fact. But it was used as a basic reference in the construction of those parts of the McGovern Committee's Dietary Goals (U.S. Senate 1977) dealing with the reduction of simple carbohydrates in the American diet. A look through the Dietary Goals will uncover suggestions that sugar consumption is associated with development of heart disease, diabetes and tooth decay; that it increases the risk for obesity; and that sugar in the diet displaces complex carbohydrates and other nutrients as well.

The Dietary Goals were first published early in 1977 with a backdrop of several years of hearings. It is of considerable interest to note that by December, 1979, at least in government circles, the association of sugar consumption with health and nutrition problems had been reduced to one, namely, tooth decay. This is brought out in the position paper on Food Labeling (Fed. Regist. 1979), produced by the FTC/USDA/FDA, in which it is stated that "...the only demonstrated hazard that sugars pose to public health ... remains their contribution to dental decay." This is a truly amazing decrease in the number of sugar-health associations within a relatively short period of time. The major reasons would seem to have been (1) the evaluation of the status of sugar in the diet by several groups of scientists along with (2) a concerted effort by the sugar and sugar-user industries to put the facts before the public and, where information was not available, to (3) generate the facts through a sound research program.

The panel evaluation I allude to included the review, Sugar in the Diet of Man (Stare 1975), the so-called FASEB report on Sucrose (U.S. Dept. of Commerce 1976) and the report of the American Society for Clinical Nutrition Task Force (Bierman 1979). These reviews reflected the interest in the problem by scientists and government agencies. Industries' response to the health questions about sugar can be found not only in the publicizing of known facts but in the development of a vigorous research program during the 1970's specifically designed to generate answers to sugar oriented medical and dental questions. The results from several of the research projects sponsored within this program were presented at a symposium entitled "Sugar and Health," at the 41st Annual Meeting of the Institute of Food Technologists, June 8, Atlanta, GA. The symposium offered a good cross section of the type of research we have supported.

You may well conclude from the papers given at the symposium plus the evaluations of sugar-health associations by expert

scientific panels and the Food Labeling position paper cited that the role of sugar in the diet is in much more proper perspective than it was in the early 1970's.

Certainly for health problems where attempts to associate sugar have been strongest - heart disease, diabetes, obesity and dental caries - the picture has come into focus. In the development of heart disease, prospective studies such as the Puerto Rico Heart Health Program (Garcia-Palmieri et al, 1980) continue to show that dietary sucrose intake by a free-living human population shows no relationship to coronary heart disease incidence.

In the development of diabetes, genetics seem to play a basic role, superimposed with environmental factors (Fajans 1981) and, while the American Diabetes Association (1979) recommends restricting mono- and disaccharide intake by adult onset diabetics because of potentially induced hyperglycemia, there is apparently precious little evidence in the literature to support this regimen (Arky 1978).

Obesity is a severe problem in the United States. It appears difficult to identify why it prevails and what to do about it. Current theories suggest that if sugar is involved in the development of obesity it is only because, like other dietary constituents, it is a calorie source and not because it is sucrose, per se (Van Itallie and Hirsch 1979).

Even in the field of tooth decay, wherein sucrose admittedly plays a role, the profile of sugar has been reduced significantly as the importance of other ingredient factors such as nutritional status, trace elements, and eating habits have been given more attention (Forsyth Dental Center 1979).

And so the scientists, industrialists, government representatives and even some media representatives (Anon. 1979) during the 1970's have modified their positions so as to reduce associations of sugar with health and nutrition problems.

CONSUMER AWARENESS

But how about the consumer? The Food Labeling position paper (Fed. Regist. 1979) includes a report of results from a consumer survey conducted by the FDA. Among the findings from this survey is the apparent fact that within that small segment of the general public who care what goes on a food label, the major concern is over sugar content. And we do not believe that the public concern over sugar content of foods is restricted to the involvement of sugar in tooth decay as the food labeling paper might imply. Rather we feel that this concern evolves from the belief, however generated, that sugar is indeed associated with the development of heart disease,

diabetes, obesity, hypoglycemia, hyperactivity, hypertension, food sensitivities, juvenile delinquency and more.

The efforts of the sugar and sugar user industries to publicize facts and to generate answers to questions on sugar and health plus official positions taken by scientists and government agencies were in part, at least, responsible for a quietus in assaults against sugar during the latter part of 1979 and the early part of 1980. On the other hand, perhaps there was recognition on the part of the media that the public was still not convinced of the absence of associations between sugar and health or nutrition problems. This media reading of the consumer may be responsible for a renewed promotion of these suspected associations.

RENEWED ANTI-SUGAR ACTIVITY

No matter the reason, both the quietus and renewed activity have occurred. And such renewed activity is to be found among consumer advocates, all parts of the media, governmental agencies and scientists.

For instance, advocate actions include a calling for a re-examination of sucrose as a GRAS substance by the CSPI (Nutrition Action 1980), Prevention magazine's listing of sucrose as the worst food component (Anon. 1980) and the production of a film by Pyramid (1981) depicting the "harmful effects of sugar on the physical and mental health of you and your children."

USAir, in its March 1981 magazine published an article entitled Sugar and Your Health in which the amount of sugar consumed was misquoted, the implication was made that sugar consumed with fats promoted heart disease and generally recommended cutting down on dietary sugar.

We find government activities also moving against sugar usage. The USDA in particular has been instrumental through regulations in halting the sale of sugar-containing products during school lunch hours (Fed. Regist. 1980B). They have also caused elimination from WIC programs any dried cereal containing over 6 g of sugar per serving (Fed. Regist. 1980A).

Add to these the fact that both the FDA and National Cancer Institute have requested that sucrose be included for carcinogenicity testing in the National Toxicology Program (USDHEW 1980) and we can see that all anti-sugar sentiment does not lie with advocates and headline hunters.

Some scientists also still make associations between sugar and some health problems. The American Journal of Clinical Nutrition has published articles suggesting a relationship of thiamin deficiencies to intake of high sugar containing foods

(Lonsdale and Shamberger 1980). Recent articles in the same journal call attention to a possible correlation in some developing countries of intake of high sugar containing foods with malnutrition (Desai et al. 1980). The AJCN has also recorded experiments on monkeys, results from which suggest that sugar has a synergistic effect on salt in the production of hypertension (Srinivasan et al 1980).

Finally, the Journal of Nutrition over the past several years has carried many articles from Dr. Reisner's labs recommending attention to carbohydrate sensitive people, the disaccharide effect and the potential involvement of sugar in the development of heart disease and diabetes (see for instance, Hallfrisch et al 1981 and Chapter 11, Food Carbohydrates).

These anti-sugar activities have been purposely overemphasized in order to point out the necessity of continuous monitoring. There are indeed many scientists who find no correlation of sugar intake with diabetes and heart disease. There are media representatives who are not violently anti-sugar and have been willing to say so through their respective media. There are Congressional staffers and agency personnel willing to incorporate data on sugar supplied by the industry. There are reporters who have treated the subject objectively and even some advocates with whom we can enter into a nonviolent conversation.

But the big points are that despite the strong, well directed and well founded efforts by the sugar industry to keep people properly informed (a) unless the consumer is contacted in an understandable and credible manner and (b) unless there is an ongoing curiosity and programmed research designed to address questions regarding sugar and health, the presentations of those dealing primarily with opinion will hold sway. We feel that the same points apply to any segment of the food industry and not to sugar alone.

ADVANTAGES OF SUGAR

We have often been chastized for being completely defensive on the subject of sugar and health and urged to walk on the sunny or positive side of the street. So let's point out some of the positives for sugar in nutrition and health by citing some current uses for sugar throughout the world and by describing some of the research we are actively supporting.

Much of the use of sugar in medical areas, worldwide, is to be found in the lesser developed countries rather than the developed ones. For instance, in countries where dehydrating diseases such as cholera prevail, sugar plus electrolytes are a preferred treatment in order to rehydrate the subject and re-establish the electrolyte balance (Nalin et al, 1978).

In several countries in South and Central America where there are many cases of blindness due to lack of vitamin A in the diet, it is the law that all sugar sold within the country must be fortified with vitamin A. A recent report tells of the success of this program (Arroyave et al, 1979).

Again following the lead that a well nourished mother will give birth to healthy and good weight children, sugar is found to be a cheap, available calorie source for pregnant women (Lechtig et al, 1979).

At home a somewhat curious experience is being recorded in the South where it has been reported that the use of sugar on open wounds and severe burns aids in the healing process (Knutson 1980).

CURRENT PROGRAM

As part of our research program we are deeply involved in helping to establish methods for determining the relative cariogenicity of foods.

We have also sponsored research the results of which have shown that sucrose along with other carbohydrates in the diet promotes the formation of the brain neurotransmitter, serotonin, which, in turn, seems valuable in assisting behavioral controls over such activities as appetite, activity and learning ability. In addition we have been involved in the estimation of the value of sucrose in the diets of long distance runners.

All of these subjects were dealt with in detail at the IFT. Symposium previously mentioned.

But we have not stopped there. We are currently sponsoring research designed to establish whether or not salt in the presence of sugar causes greater hypertension than salt alone. We have started a program which we consider will show how much sugar can be incorporated into the diets of adult onset diabetics. And we will soon be deeply involved in trying to answer the question as to whether or not sugar in the diet will accentuate hyperactivity in children identified as hyperactive.

We look on all these projects as being well targeted to answer questions posed about possible effects of sugar in our diet as well as to develop very positive aspects about the product.

SUMMARY

In summary, I have tried to give, briefly, many concepts, facts and opinions regarding sugar and its role in health and nutrition. I want to leave you with the distinct impression that the sugar and sugar user industries have, along with a

natural vested interest, a decidedly deep concern for the subject, that we have dealt with the problems thoughtfully and forthrightly and will continue to do so in the future. I believe that by our experience other parts of the food industry can profit.

Should one of your products come under attack in health and nutrition areas perhaps you will find as we have, that the following steps can be helpful in countering such attacks:

1. Find out the status of things; do a literature search.
2. To help deal with questions not answered in the literature set up a sponsored research program. Have the research address specific targets.
3. Develop good communications systems such that information searched out and generated through research will reach the consumer in an understandable and believable manner.
4. As your information and communications system unfolds, make an effort to detect which segments of the population you have yet to reach and double your efforts to do so.
5. Continuously monitor new developments in the field whether scientific, political, advocacy or renewal of former attacks.
6. Having built a reliable defence for your product, promote its good points to the same audiences.

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DISCUSSION

J. F. DOWLING (Refined Sugars): We always hear that people eat too much sugar. Suppose we take the other approach and assume that very little to almost zero sugar was consumed. What would be the effect of such an extreme situation?

G. N. BOLLENBACK: It is theoretically possible for someone to exist without consuming any sugar at all, but nobody is going to do that. People are going to eat sugar. Even the most avid anti-sugar spokesmen admit that. They have to try a different approach than trying to eliminate sugar from the diet completely. There are some countries in the world that have a very small per capita consumption of sugar, at least from a statistical point of view using known sources. However, I have seen in several of those countries that there are many primitive mills around that produce pinella or guhr type sugar that does not get counted in the statistics. So, you don't really know the true consumption of sugar. These countries with very low consumption are exclusively the third world or developing countries or underprivileged or poorer countries. They have a problem of malnutrition due to the lack of other things but mostly calories. In India, protein is not a problem; calories is the problem. In regard to tooth decay, obesity, heart disease, in those countries with very low sugar consumption there are so many things that prove fatal before these can develop that it is very hard to tell.

F. W. PARRISH: One approach in dental caries protection would involve immunization. What are the prospects for achieving this in this century?

G. N. BOLLENBACK: So far as immunization is concerned, if the National Institute for Dental Research (NIDR) had its way, we would be testing what they have developed for immunization right now. They have worked with monkeys and developed an immunization technique. It involves taking encapsulated streptococci and swallowing them. They claim that they have had a decrease in potential caries for people that did that. These are not children but older people. Even though a process for immunization may be developed at NIDR, they have a number of years before clearing it with FDA.

F. W. PARRISH: In connection with diabetics and the need for them to know the sugar content of foods plus the fact that labeling research information is very costly in the food industry which has a low profit margin, what would you advise in connection with that?

G. N. BOLLENBACK: There is a lot of voluntary labeling of sugars, especially on the cereal boxes. There is a report on sugar labeling by a USDA, FDA, FTC task force that has been on hold for about a year that would require sugar labeling. This is dormant until sugar gets higher on the priority list for labeling, and that will not come for another year. Possibly it will not come within this administration. The labeling is going to be proposed to include such things as using the term "sugars" as a generic term for nutritive sweeteners; with then, as they do in Canada, a parenthetical explanation of what the sweeteners are. The FDA proposes to go a step further and include in that term, sugar alcohols, so that sorbitol, manitol and xylitol would be included in the generic term "sugars". What the trigger level of sucrose content would be has not yet been decided. They don't have any good basis to make that decision because the scientific background for doing any labeling is tooth decay. It isn't diabetes or heart disease. The only thing that we have in that field is a guess that 6 grams per ounce should be the trigger point.

SCIENCE AND THE ART OF LOW PURITY CANE SUGAR CRYSTALLIZATION

Elmer J. Culp

Consultant

INTRODUCTION

The greatest loss of sugar in the refinery occurs in the production of final molasses. Given that cane sugar molasses tends to run about 40 true purity, sugar technologists expect that each pound of non-sucrose in the molasses will take with it about two-thirds pound of sucrose. And yet -- there is enormous potential for greater yields to be obtained through reductions in the purity.

A refinery producing final molasses at 40 purity can, realistically, aim for a reduction to 35 purity. With an annual melt of one billion pounds of 98°Pol. raw sugar, the increased sugar yield would amount to about two million pounds with an annual saving (at current prices) of roughly \$300,000. It is surprising that -- faced with this potential -- the cane sugar industry has sponsored so little research covering low purity crystallization.

Over the years, refiners have developed -- primarily through trial and error -- practical methods of achieving acceptable sugar extraction. Some of our better known technologists (Gillet 1977, Weber 1977) have published many "rules of thumb" that are widely used for day-to-day process control. On the other hand, very little scientific research has been done on low purity crystallization. There is much truth in the surmise (VanHook 1980) that "established practices may not be optimal".

VanHook mentioned the "sparsity of published data", and he stated that "The chief deterrent to detailed application at this time . . . is the dearth of appropriate published data". Recognizing this fact, the author of this paper has searched for the pertinent information that is available. The primary purpose of the paper is to interpret the scientific facts as they apply to actual operating experience.

The relationship between theory and practice that will be set forth here makes use of a fortuitous coincidence in the published data. It involves solutions in which the invert content comprises 35% of the non-sucrose solids. It also depends upon the relationship published recently (Culp 1981) in which maximum rate of low purity crystallization for beet sugar is achieved with about 4.5 molecules of water present per molecule of sucrose.

SOLUTIONS IN WHICH INVERT COMPRISES 35% OF NON-SUCROSE SOLIDS

Molasses Purity Formulas

Many formulas have been proposed from time to time for prediction of the purity of well extracted molasses. The following two examples are rather well known (Lyle 1941 and later) and are of particular interest for the purposes of this study:

$$TS = 55 + 14g \quad (1935 \text{ Formula})$$

$$TS = 100 \frac{(1.4 + \frac{g^2}{2})}{(2.5 + \frac{g^2}{2})} \quad (1944 \text{ Formula})$$

Where TS = % total sugars, solids basis
and g = ratio: invert/non-sugars

With appropriate mathematical treatment, each of these two formulas yields an expression for the ratio of sucrose to non-sugars as a function of g. The results are shown in Figure 1.

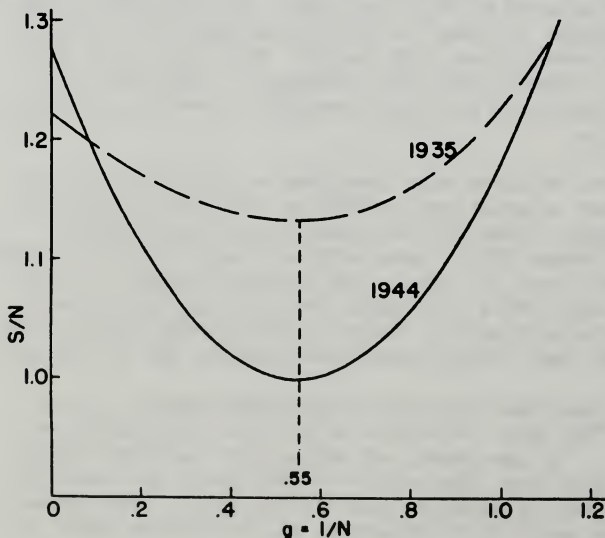


Figure 1. -- Predicted Sucrose/Non-sugars Ratios

The slope of each curve represents the incremental value of invert's melassigenic influence. It is significant that both of the curves have minimum points ($g = .54$ for 1935, $g = .55$ for 1944) and that these points fall at practically the same value of g . The slope to the left of the minimum is negative, corresponding to the well known "salting out" effect of invert. To the right it turns upward, showing the melassigenic influence at the higher invert concentrations. At the minimum point the incremental additions of invert have just spent all of their "salting out" potential, so the corresponding value of g represents a unique physical state.

It seems to be very significant that the two formulas that were developed by Tate & Lyle for an entirely different purpose confirm each other so well at the minimum points of their curves. Incidentally, the critical values of g are equivalent to 35% invert in the non-sucrose solids, and it is this value that has been used in calculations for the rest of this paper.

Solubility Relationships

Solubility data for low purity cane sugar solutions have been published (Charles 1977) in the form of curves on a graph. Apparently the molasses component of the solutions used for the solubility measurements had the following approximate composition:

	<u>% of Solids</u>	<u>% of N.S.S.</u>
Sucrose	44.06	--
Invert	19.55	35
Non-sugars	<u>36.39</u>	<u>65</u>
Total	100.00	100

It is an interesting coincidence that this particular molasses contained the same amount of invert that was found earlier in this report to be a critical melassigenic point. This fact is to be kept in mind while looking for additional scientific information that can be derived from the Charles solubility curves.

Recently it was pointed out (Culp 1981) that a saturated pure sucrose solution's concentration is linear with temperature ($N_C = 10.65 - .0674T$) when it is expressed as the molecular water/sucrose ratio. Based on Charles' data, Table 1 lists the number of water molecules per sucrose molecule at saturation, for selected levels of temperature and purity. Figure 2 shows the same information in greater detail.

Table 1.--Solubilities in Terms of N_S

Temperature °C.	100 Purity	80 Purity	60 Purity	40 Purity
20	9.5	10.4	11.7	14.7
40	8.1	8.8	10.0	12.1
60	6.6	7.1	8.0	9.3
80	5.1	5.4	5.9	6.5

Clearly, the saturated cane sugar solutions have progressively higher molecular water/sucrose ratios at the lower purities.

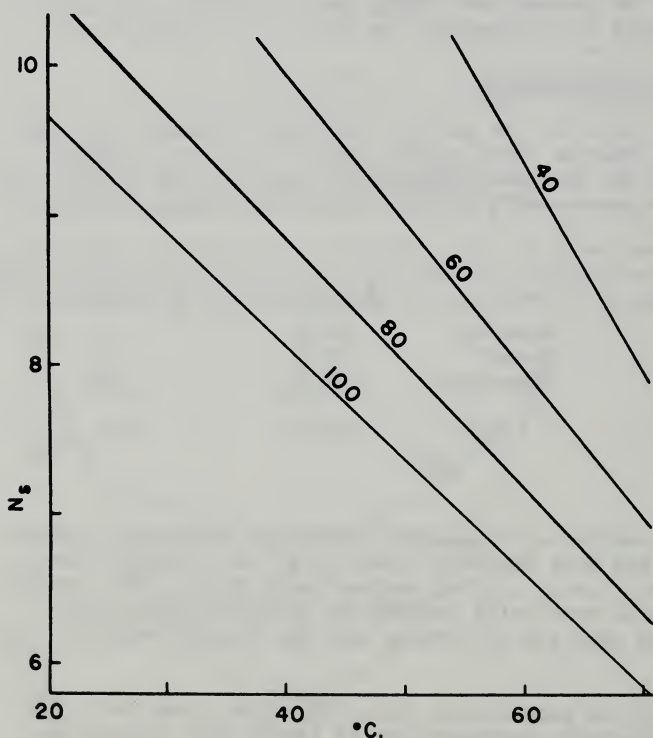


Figure 2.--Solubilities in Terms of N_S

Recalling that the "35% invert" molasses has unique melassigenic properties, it occurred to this writer that it might also have unusual solubility characteristics. Accordingly, the solubility data already shown have been modified. Instead of using N_S to represent concentration, the term " N_T " has been substituted. As applied here, N_T is the number of water molecules per molecule of total sugars (sucrose equivalent). Table 2 lists the values of N_T that correspond to those of N_S in Table 1. Figure 3 shows selected N_T data for 60 purity compared to the 100 purity line.

Table 2.--Solubility in Terms of N_T

Temperature °C.	100 Purity	80 Purity	60 Purity	40 Purity
20	9.5	9.6	9.6	9.8
40	8.1	8.2	8.1	8.1
60	6.6	6.6	6.5	6.2
80	5.1	5.0	4.9	4.3

These results show that, for "35% invert" cane materials, N_T has practically the same values regardless of purity. In other words, the relationship of concentration to temperature does not change very much with purity, provided that the concentration is expressed in terms of the number of water molecules per molecule of total sugars (sucrose equivalent).

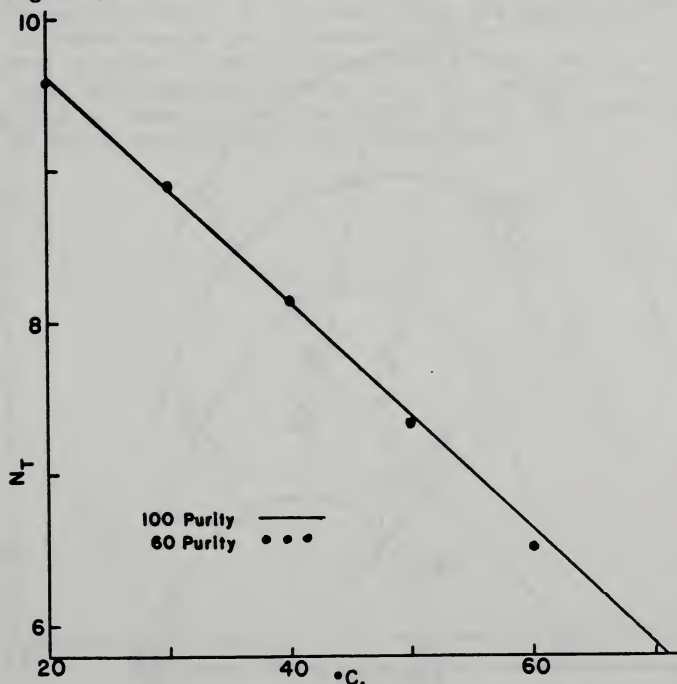


Figure 3.--Solubilities in Terms of N_T

The reason for this solubility relationship is not clear. It seems that two molecules of reducing sugar require the same degree of hydration as one sucrose molecule when influencing the solubility of sucrose.

RATE OF CRYSTALLIZATION

Extensive measurements have been reported (Brieghel-Müller 1962) covering crystallization rates of beet sugar solutions. At each purity level, from 97.5 down, increasing concentration first resulted in faster crystal growth, while at still higher concentrations the rate actually decreased. Representative data from the Brieghel-Müller work at 50°C. are shown in Figure 4 as curves for 62.5, 65, 67.5 and 70 purity respectively. The two broken lines connect points on the curves that correspond to 4.5 molecules as well as 5 molecules of water per molecule of sucrose. It is clear that maximum crystallization rates are achieved when the molecular water /sucrose ratio is between 4.5 and 5. In private communications VanHook has stated his feeling that the 5 molecule figure can be used. On the other hand, there are theoretical reasons (to be made clear later) for using the model containing only 4.5 molecules of water. At any rate, calculations must be made with a single figure, and this writer has chosen 4.5 molecules of water per molecule of sucrose as the condition for maximum crystallization at 50°C.

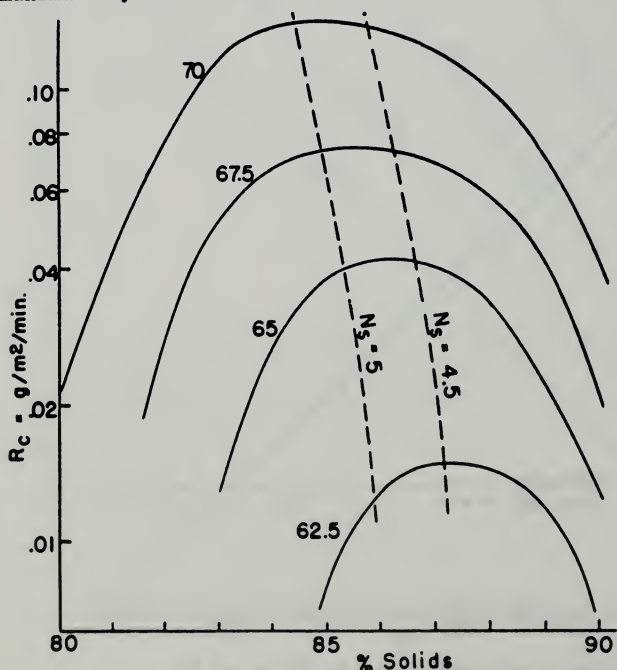


Figure 4.--Crystallization Rates for Beet Sugar Solutions at 50°C.

This writer has not been able to find published data on rates of crystallization for impure cane sugar solutions. References to this subject usually dismiss it with some statement such as "The rate of crystallization increases with higher supersaturation." On the other hand, sugar refiners who have worked with modern, high capacity equipment know from first hand experience that very high concentration reduces the rate, sometimes drastically.

Although actual rates are not readily available for cane materials, sugar refiners have practical information that was developed from the boiling of many thousands of sugar strikes. This experience has been distilled into all kinds of practical "rules of thumb", intended to maintain the maximum crystallization rate. For example, it has been reported (Lyle 1941) that, for 2nd and 3rd crop boilings, the "supersaturation coefficient" is approximately 1.3 for growing and 1.35 for dropping.

Refiners are interested in the trend of molasses purity while a low strike is being boiled. Figure 5 shows how the purity changes (heavy line) as a function of per cent solids, for a strike boiled at 150°F. using Lyle's control points. During the crystal growth period in the pan, supersaturation is maintained at 1.3. The mother liquor's purity decreases due to loss of sucrose to the crystalline phase. At the same time, its solids concentration increases because the saturated solution (so, too, the supersaturated solution) is more concentrated at the lower purities. This trend continues until the pan is full, at which time the syrup feed is turned off and the mother liquor is concentrated to 1.35 before dropping into the crystallizer.

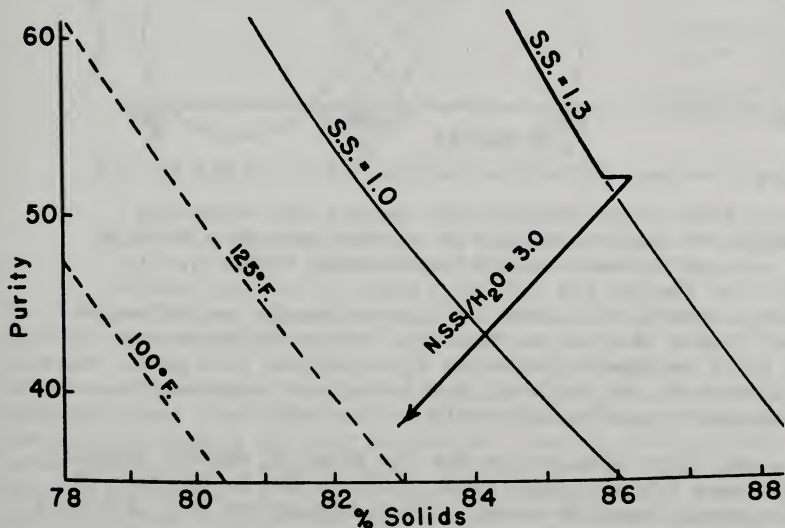


Figure 5.-- Purity-% Solids Trends in Low Purity Strike at 150°F.

Once the massecuite has reached the crystallizer, its non-sucrose solids/H₂O ratio remains constant. (In the illustration, N.S.S./H₂O = 3.0) Both the purity and % solids decrease as the crystallization proceeds, following the trend line as shown.

Since we know from refinery experience that the 1.3 supersaturation level must correspond approximately to maximum crystallization rate, it is appropriate to see whether this is close to the $N_S = 4.5$ condition that worked for beet sugar. One quickly finds that the approach does not apply to cane sugar. As shown in Figure 6, the broken line representing $N_S = 4.5$ does not come very close to the S.S. = 1.3 that was used for boiling control.

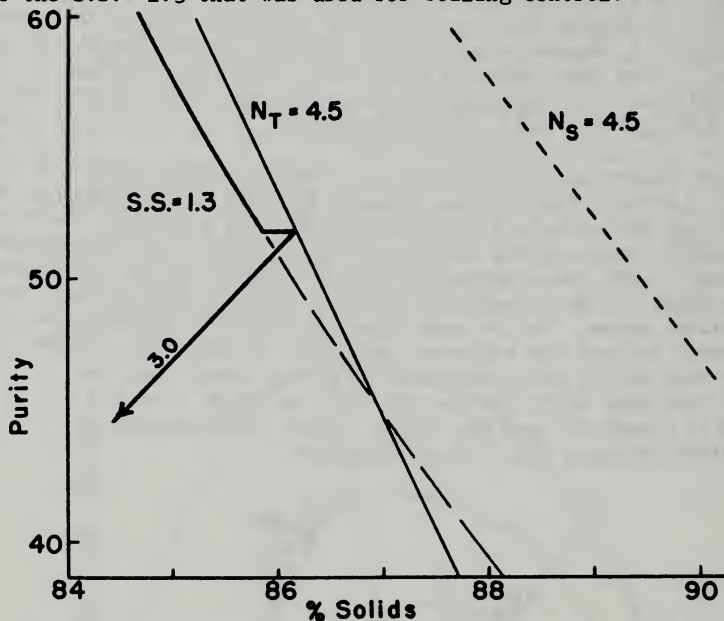


Figure 6.--Close Correlation Between S.S. = 1.3 and $N_T = 4.5$

Recalling that invert behaved like sucrose when affecting solubility, we shall next consider whether this is also true in the case of maximum crystallization rate. Specifically, we shall see whether the S.S. = 1.3 point of boiling control is close to the $N_T = 4.5$ ratio of water molecules per molecule of total sugars (sucrose equivalent). Referring again to Figure 6, it is apparent that the $N_T = 4.5$ curve is a good approximation to the S.S. = 1.3 level that has been found to yield maximum crystallization rate in the refinery.

What is the proper designation for the point of maximum crystallization rate? Historically, most refiners (as in the case of Lyle) prefer to speak in terms of "supersaturation"; i.e., S.S. = 1.3. As shown here, the same purpose would be served if we were to label it " $N_T = 4.5$ ". Even more possibilities are available. For example, cane materials that have maximum crystallization rate

at 150°F. are just saturated at about 195°F., so we could just as logically call it " $T_s = 195$ ".

The situation brings to mind an adult education course in astronomy that was coming to its close. During the period of the instructor's review, he asked for any questions that the pupils might have. A little old lady said, "Sir, I have learned that you used the interferometer to measure the stars' diameters, and you used the mass spectrometer to determine their masses. What I don't understand, though, is how you ever found out their names!"

As we have seen, the established - but arbitrary - way of designating a massecuite's mother liquor concentration is to refer to its supersaturation. It is important, though, that we remember there are other terms just as useful and, perhaps more descriptive of the true situation.

HYDRATION STRUCTURE OF SUCROSE MOLECULES IN AQUEOUS SOLUTION

It is timely for us to consider the physical significance of the $N_s = 4.5$ and the $N_T = 4.5$ expressions. Fortunately, there is available a good, published description (Schliephake 1963) of the probable molecular relationship.

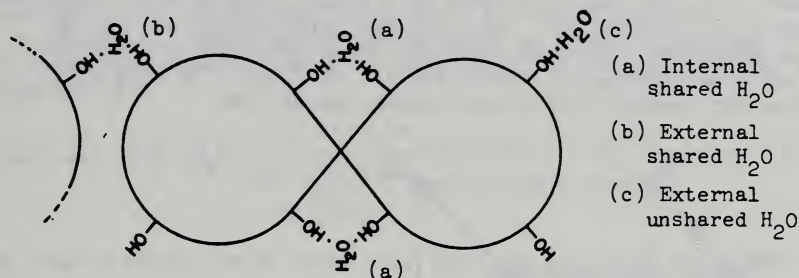


Figure 7.--Hydroxyl Groups on a Single Sucrose Molecule.
Three shared water molecules are shown.

In the case of pure aqueous solutions, one-half of the eight hydroxyl groups in the sucrose molecule are tied up by weak hydrogen bonds with two "internal" molecules of water. This situation is shown in simplified form on Figure 7. The internal bonding leaves just four "external" hydroxyl groups. These are bonded individually by water molecules. Most of the external water molecules are shared by two hydroxyl groups on adjacent sucrose molecules. When there are only enough of the water molecules to bind together all of the pairs of hydroxyl groups, the

sucrose molecules lose their mobility and cannot diffuse. Any excess water breaks the bonding pattern and permits the sucrose to diffuse.

In summary, with the $N_S = 4.5$ condition the hydration molecules are accounted for as follows:

Per Sucrose Molecule:

Internal Hydration	-	2
External, shared	-	2
External, unshared	-	<u>.5</u>

Total H_2O molecules - 4.5

Since hydrogen bonds are relatively weak, the shared water molecules do not adhere very tightly. Accordingly, the solution is analogous to a "soup" made up of hydrated sucrose clusters of varying sizes. The water molecules continually move around from one molecule to another. Consequently, when we speak of 4.5 molecules of water per molecule of sucrose we refer to some kind of statistical average.

Although the number of sucrose molecules in a cluster is believed to vary, we might visualize the general structure better if we consider one possible arrangement. Figure 8 shows how eight sucrose molecules can be grouped in a single cluster while conforming to the $N_S = 4.5$ condition. Each one has three shared and one unshared molecules. The four unshared water molecules protect the cluster from adhering to other, similar clusters. In this way the cluster remains free to move about, or diffuse.

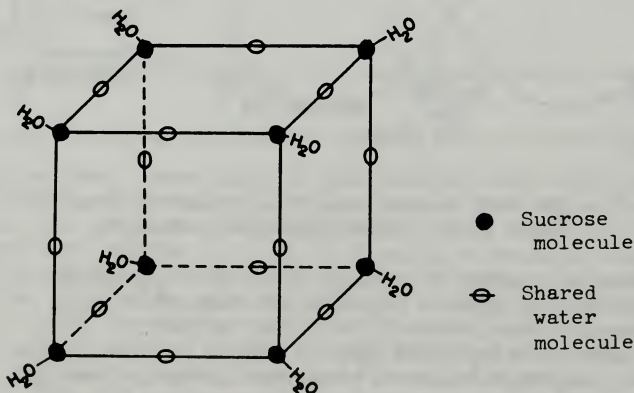


Figure 8.-- Example of Possible Structure in a Hydrated Sucrose Cluster

The shared water molecules protect the sucrose molecules from direct contact with one another. Without this protection, one or more pairs of the hydroxyl groups could bond together more tightly than they do with the water present.

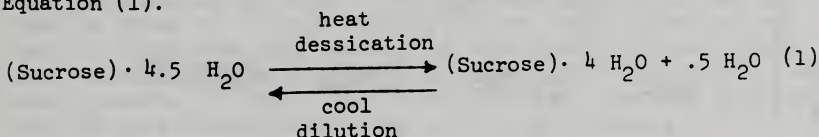
As already indicated, invert in certain cane sugar solutions (where invert = 35% of N.S.S.) tends to substitute for sucrose when influencing solubilities and maximum rates of crystallization. With such materials we have used the term N_T instead of N_S . We do not know how this substitution takes place.

EXTRACTION EFFICIENCY

Hydration Equilibrium

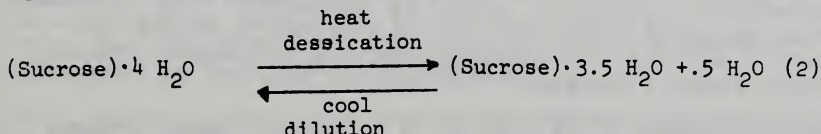
Referring again to Figure 5, there seems to be no clear reason why, with proper crystallizer treatment, the true molasses purity should not drop to the middle 30s or even lower. And yet - this kind of performance is rarely achieved in the refinery. In the light of facts already disclosed in this paper, we can now consider some of the reasons for poor performance and begin to think about possible remedies.

Let us take as an example, greatly simplified, the case of a sucrose molecule in massecuite that is boiling at its maximum rate of crystallization. As already seen, it will be associated (on the average) with about 4.5 water molecules; i.e., $N_T = 4.5$. Since the hydration water is bound so weakly, an occasional molecule tends to break away, in accordance with Equation (1).



As long as there are at least four exterior hydration molecules, each sucrose molecule is protected from direct contact with others and the reaction can take place rapidly in either direction.

As a further example, let us consider what happens to the sucrose molecule studied above when $N_T = 4$. The simplified reaction is represented by Equation (2).



As before, this reaction proceeds rapidly toward the right. It can take place only by stripping an external hydration molecule from a sucrose molecule, leaving a hydroxyl group exposed. This

allows two sugar molecules, so stripped, to link together forming a bond between two hydroxyl groups that is stronger than the weak hydration bond. For this reason Equation (2) proceeds very slowly toward the left.

The Problem Areas

The practical significance of Equations (1) and (2) will become clearer through consideration of Figure 9. It shows curves for $N_T=3.5, 4$ and 4.5 , respectively, using the same purity and % solids coordinates that were employed for Figures 5 and 6. The $N_T=4.5$ curve falls mostly in the 85% to 87% solids range that is consistent with normal sugar boiling conditions. On the other hand, the $N_T=3.5$ curve ranges around the 90% solids level. Many sugar refiners have experienced "refractory" syrups and high molasses purities when their final remelt massecuites contained molasses with such a high concentration.

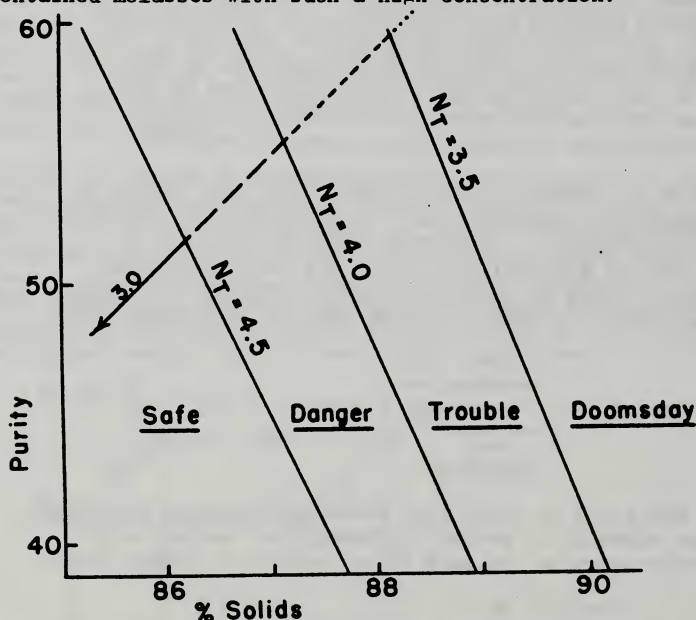


Figure 9.-- The Problem Areas

The area to the left of the $N_T=4.5$ curve is relatively free from undesirable formation of refractory substances. There are enough water molecules to surround each cluster and to protect the sucrose crystals from direct contact with one another. Accordingly, Figure 9 designates this area as "Safe".

The area between $N_T=4.5$ and $N_T=4$ is labeled "Danger". Syrups within this zone contain some sucrose clusters that are not protected by unshared water molecules. Such sucrose is vulner-

able to loss of a shared water molecule during continued boiling, as in Equation (2). There is some tendency toward direct bonding of adjacent sugar molecules.

The term "Trouble" is used between the $N_T = 4$ curve and the $N_T = 3.5$ curve. Materials in this range react rapidly according to Equation (2) while they are being subjected to the boiling conditions that exist in a vacuum pan. The reaction is practically irreversible under normal operating conditions.

To the right of $N_T = 3.5$ is the term "Doomsday". Once this area has been reached the damage has already been done. The molasses has become "refractory"; i.e., the sugar is very difficult to crystallize.

One person might say that refractory syrups have merely become too concentrated or viscous for efficient crystallization to occur. From another view, one might find that the real purity has dropped markedly due to direct bonding of some of the sugar molecules. (Conventional laboratory test methods would not show this, for they involve substantial dilution of the samples.)

To summarize the situation, it seems clear that low purity solutions should always be boiled as close to $N_T = 4.5$ as practicable. Concentrations higher than that will result in slower crystallization and in poorer sugar extraction.

In accordance with the position taken elsewhere in this report, it should be understood that invert acts much the same as sucrose when influencing the latter's maximum rate of crystallization. When we speak of "direct bonding" of two sugar molecules, we mean to imply that such bonding can take place between sucrose and reducing sugar molecules. Since beet materials contain such small amounts of invert, the principles that have been proposed may not have direct application in the beet industry.

A WORD ABOUT VISCOSITY

Much has been written about the viscosity of low purity cane materials. Usually, however, the subject terminates with the statement that viscosity depends upon the "nature of impurities present". Surely this view has justification, particularly after all of the work that has been reported on dextran and its adverse influence. This report does not refer to the kinds of abnormal impurity that are exemplified by dextran. Instead, it is intended to cover the normal cane sugar materials that are encountered by refiners in their day-to-day operations.

It is true, of course, that lower purity molasses may have higher per cent solids. For this reason, its viscosity can also range higher. This is a result - not a cause - of the low purity.

Molasses can have high viscosity for three reasons:

1. Low purity, with accompanying high per cent solids.
2. Presence of an abnormal impurity, such as dextran.
3. Direct bonding of sugar molecules.

The third cause is controllable by the refiner. Heretofore, such control has been impeded by scarcity of sound scientific information. Lacking a better basis for process control, technologists have been forced to rely on the "rule of thumb" approach.

SUMMARY

Based on the limited, existing scientific knowledge, the lines of reasoning presented in this paper constitute a rational approach to efficient, low purity cane sugar crystallization. When excessive concentration occurs under conditions that exist in the vacuum pan, bonding of the sugar molecules takes place. Crystallization rate becomes slower and viscosity increases. By stopping just short of such excessive concentrations, the refiner can maintain maximum rates of crystal growth while minimizing the harmful reactions.

On the basis of this information, one is now able to formulate suitable answers to the following questions:

1. Why is low temperature better than high temperature and controlled concentration better than higher concentration for low purity remelt boiling? - Because it is only in this way that efficient crystallization can be maintained, through avoidance of "refractory" molasses with its excessive viscosity.
2. Why is recycling of low purity syrups harmful? - Because longer exposure to high temperature and high concentration promotes the harmful "refractory" condition.
3. Why is it sometimes necessary to discard the higher purity remelt syrups to molasses production and to "start over" with fresh materials for remelt boiling? - To eliminate from the system materials that have been boiled too hot, too heavy and too long.
4. Why is refinery molasses purity higher than that in a raw sugar factory? - Because it contains substances that have been heated longer and boiled more times.
5. Why are low temperatures necessary for production of good soft sugars? - To minimize the harmful effects of boiling and recycling the syrups, thus avoiding development of high viscosity that impairs the soft sugar texture.

6. Why does newer, more modern equipment sometimes become involved with poorer extraction efficiency?-- Because it is difficult for refiners to avoid the temptation to boil the massecuite to excessive concentration and to use higher steam pressures.
7. Is low purity crystallization theory applicable to conditioning of white refined sugar and of soft sugar?-- Yes, for in both cases the objective is to achieve maximum crystallization of sucrose from the surface film, with release of bound moisture.
8. Is there a difference between cane sugar and beet sugar operations in their low purity extraction?-- Yes, the fundamental distinction involves lack of significant invert in the beet sugar solutions. We have seen that invert can substitute for sucrose in processes involving the latter's solubility and crystallization rate; also, it can bond with the sucrose to form viscous, non-crystallizable substances.

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DISCUSSION

J. F. DOWLING (Refined Sugars): You developed a number of interesting relationships that exist when the invert content is equal to 35% of the non-sucrose solids. Do you have any thoughts about what would happen when invert is higher or lower?

E. J. CULP: When we consider the set of curves that came from the Tate and Lyle equations, along with the Charles analysis of low invert molasses, we find that the "35% Invert" molasses contains about one molecule of reducing sugar and one molecule of ash (as K_2SO_4) per molecule of sucrose. In other words, there is circumstantial evidence that it is a rather definite entity. Now at lower levels of invert, we seem to have a simple mixture of this "35% Invert" material with "beet-like" molasses that contains essentially no ash.

At the higher invert levels, we seem to have a simple mixture of the "35% Invert" material with pure invert molasses. (According to NBS Circular C440, exhausted sucrose/invert solutions contain 4 molecules of reducing sugar and 10 molecules of water per molecule of sucrose.) So, as I see it, the high invert molasses is a blend of this material with the "35% Invert" and the low invert molasses is a blend of the "35% Invert" with "beet-like" (invert-free) material.

W. R. TUSON (Colonial): You show that the maximum rate of crystallization would appear to occur when the ratio of water to sucrose is 4.5. Do you have thoughts about the reason for this?

E. J. CULP: Based on the work of Schliephake, it seems that dissolved sucrose tends to form hydrated clusters, with four water molecules for each sucrose molecule. If the ratio of water to sucrose is exactly 4, the system will consist essentially of one gigantic cluster. It will be ready to crystallize, but the cluster will be so large that it cannot migrate to the surface of a seed crystal. Now, if there is about one-half molecule of additional water present, this will allow the larger clusters to be broken up into smaller ones. These can then move just enough to get to the crystal surface. This thought is consistent with H. E. C. Powers' movies that show the crystal growing in spurts. Each spurt is another cluster that is crystallizing.

M. A. CLARKE: Have you extended any of these calculations to the situation in a raw sugar factory where there is less sugar and more non-sugars in solution?

E. J. CULP: One of the final points in my paper covers why we get higher purity molasses in a refinery than in a raw sugar factory. The over-all proportions of the invert and non-sugars

are about the same. The primary difference is that by the time these materials leave the refinery in the molasses, it has been cooked and recooked too many times. The resulting condensation products are "refractory"; that is, they resist crystallization. As my paper shows, it doesn't have to be that way if we just control boiling to avoid the problem areas.

MINOR CONSTITUENTS OF SUGAR AND FILTRATION

Galoust (Alec) M. Elgal

U.S. Department of Agriculture

INTRODUCTION

The filtration process in sugar refining has been studied as influenced by minor constituents in sugar. Emphasis in this study has been the effects of starch and dextran which affect filtration more than the other minor constituents. The two principal effects are filter plugging and resistance to mass transfer through the filter. Filtration parameters based on permeation have been used for the study of conditions resulting from raw sugar contaminated with excessive minor constituents. Since each sugar refinery operates at its own normal conditions and uses equipment different from another refinery, the permeation relative to the normal conditions is used for the study of deviations from the normal.

Temperature and duration of heating were used as test condition variables to observe their influence on viscosity, solubilizing and filtration rates.

MINOR CONSTITUENTS OF SUGAR

The impurities in raw cane sugar which interfere with filtration include: starch, dextran, cane wax, cane plant particles, silica, ash, residues of bacterial growth, and foreign substances (e.g. kernels of corn and animal hair).

Two of these constituents which have a lesser effect than the others because of their larger physical size are the cane plant particles and foreign substances.

Starch is the most common polysaccharide which occurs in various quantities in cane sugar. Some varieties of cane contain as high as 2% (2000 ppm) starch. The customary starch content of raw sugar is only about 0.002% (2 ppm) (Cane Sugar Handbook, Ch. 2). The presence of starch increases the sugar solution viscosity, forms a particulate dispersion which plugs

the filters and increases the frequency of filter cleaning and maintenance.

The dextrans have a molecular weight of up to millions and consist of lengthy polymers of glucose. The concentration of dextran can be as high as 3% in raw sugar. These polymers are formed by bacteria from the soil when the skin of the cane stalk is bruised, e.g. during cane cutting. Dextrans increase the viscosity of the sugar solution and thus impede the fluid flow through the filters.

The quantity of wax in sugar cane is about 0.1% on the weight of sugar and sugar is approximately 10% of the cane. Since approximately 1% of the weight of sugar cane is mud, the composition of the mud filter cake can be as high as 1% waxes and gums.

In addition to the above minor constituents small black particles were sometimes observed in the raw sugar as well as in the affined (centrifuged) sugar. These black particles were in the size of "tea leaves" (1 to 3 mm). They were probably remnants of burned cane which persistently adhered to sugar crystals and ended up in the raw sugar as shown in Figure 1.

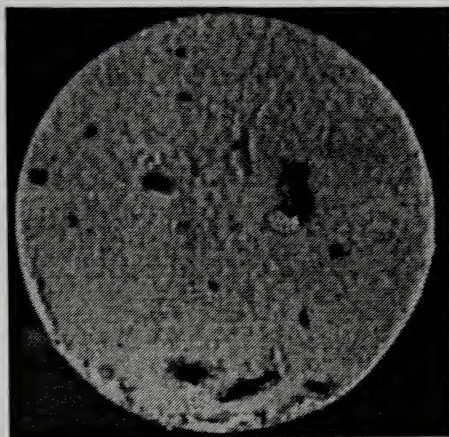


Figure 1. Particles the size of "tea leaves" in raw and affined sugar from burned sugar cane (1 to 3 mm).

EFFECT OF STARCH AND DEXTRAN ON VISCOSITY

The resistance to permeation of fluids through filter cake can be caused by two factors: physical restriction in flow passages and viscosity. The restriction of passages can be accounted for with concentration of particulate (e.g. starch) in the filter cake. Viscosity data can be used in flow equations to compute permeability.

Viscosity measurements were taken utilizing two different types of viscometers and the results were combined and checked. The measurements of viscosity versus temperature are plotted in Figure 2. All data were for 65 brix and data from the Cane Sugar Handbook (p. 839) are seen to agree with our measurements.

To determine the effect of starch heated to 80°C for one hour (simulating refinery conditions) 1% starch on weight of sugar was prepared and the viscosity versus temperature was measured as shown in Figure 2. At a temperature of about 80°C the viscosity of a 1% starch solution is about 20% above that for pure sucrose; consequently in ordinary circumstances where the starch content encountered in raw sugar is in the range of 0.002 to 0.02% the effect of viscosity contribution of starch is insignificant. The starch used was reagent grade, derived from corn with a particle size range of 5 to 30 microns, maximum 0.45% protein and maximum 0.04% fat.

Two types of dextrans were tested: commercially available dextran with molecular weight of 2 million and native (indigenous) dextran which has a varied composition of 1 to 3 million molecular weight (Imrie and Tilbury, 1972). Since, native dextran is rarely available and then only in small quantities for testing, the commercial dextran can be substituted and the data extrapolated to native dextran. At a temperature of 80°C the viscosity is 100% higher in magnitude for 1% content commercial dextran when compared to pure sucrose. When native dextran is used the viscosity is increased to 130% above refined sugar solution (Figure 2).

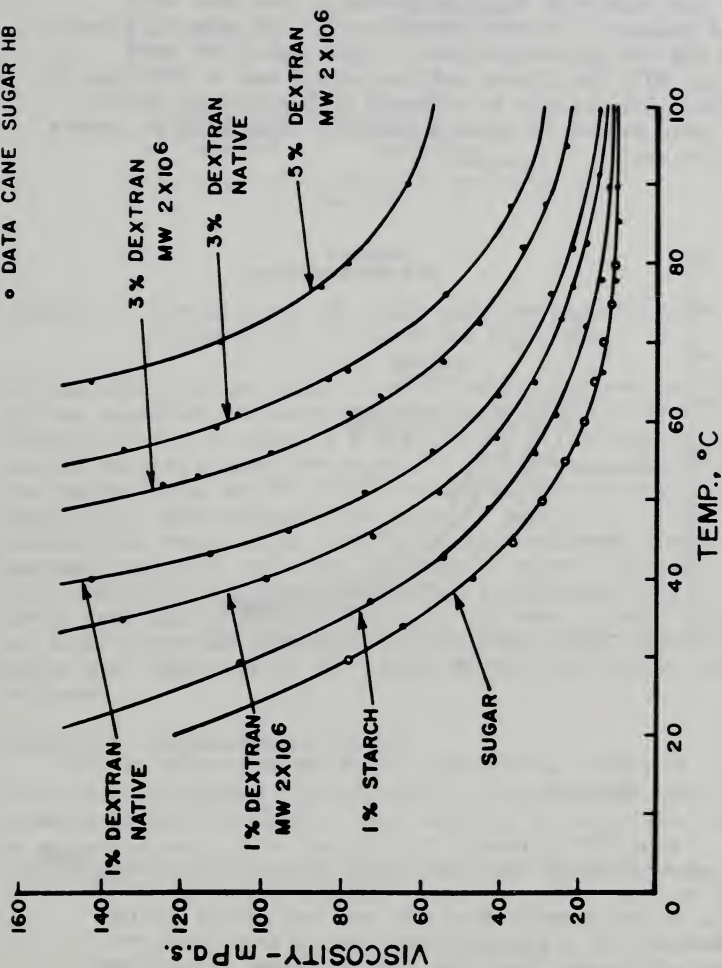


Figure 2. Viscosity comparison, 65% sugar solution

Dextran can occur to as high as 3%. For the commercial dextran the viscosity was 250% higher than pure sucrose in the 80°C range. For 3% native dextran the viscosity was 350% higher than pure sucrose solution.

When both 1% starch and 1% dextran are present, as shown in Figure 3, the viscosity is slightly lower than when only dextran is present. This is attributed to the molecular interaction of the two polysaccharides. These solutions were preheated at 80°C for 1 hour and the starch was in the form of gel granule. Gel granule is referred to particulate starch which has been heated in aqueous solution causing it to absorb water and to swell.

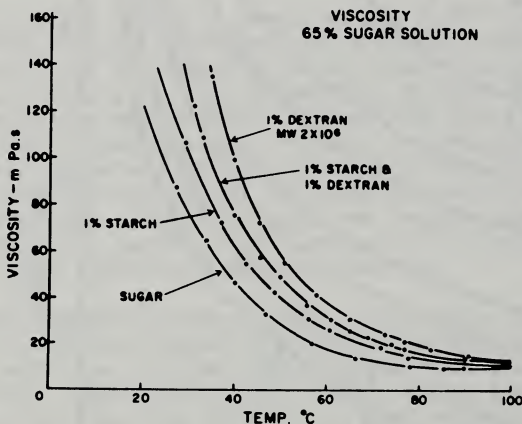


Figure 3. Viscosity of 65% sugar solution containing starch and dextran.

In a raw sugar factory the temperature of syrup may reach 100°C for about an hour. Some of the indigenous sugarcane starch may remain in the granule form and some may become solubilized. However, in a hypothetical case, starch could be heated for sufficient length of time to become solubilized, which would prevent clogging of the filters. The trade-off would be an increased viscosity (or decreased permeability) by a magnitude of 100% in the 80°C operating range as shown in Figure 4.

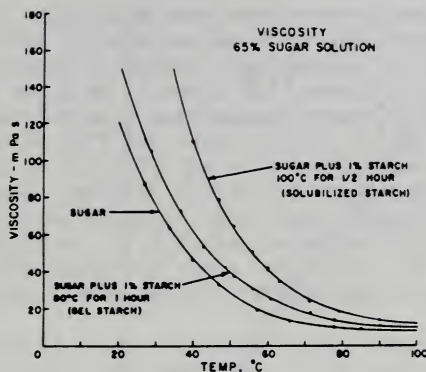


Figure 4. Viscosity of 65% sugar solution containing starch, preheated for 1/2 hour and for 1 hour.

During filter system simulations it was considered desirable to use sometimes raw sugar and sometimes refined sugar. The question arose if there is a significant difference in viscosity between a good raw sugar and pure sugar solutions. In the narrow range of 35° to 50°C measurements of viscosity were made and a least-squares line drawn by computer. It was concluded that there is no significant difference in viscosity between very good raw sugar and refined sugar (Figure 5). This confirms previously reported data (Hirschmuller, 1953) that impure sucrose solutions can have either a higher or lower viscosity than pure sucrose solutions. The differences were small except for syrups of very low purity such as molasses.

CENTRIFUGE SEPARATION OF STARCH

Centrifuge equipment was utilized to determine the temperature conditions and duration of heat required to solubilize starch. A series of tests were run with 1% starch in 65% sugar solution. The centrifuge conditions were 16,000 g's for 10

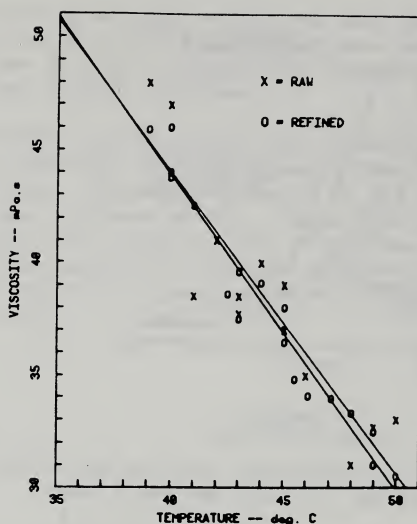


Figure 5. Viscosity comparison of raw and refined sugar.

minutes. It was observed that a minimum of one-half hour at 100°C was required to solubilized the starch; no centrifuge precipitate of granule starch was observed in these conditions (Figure 6).

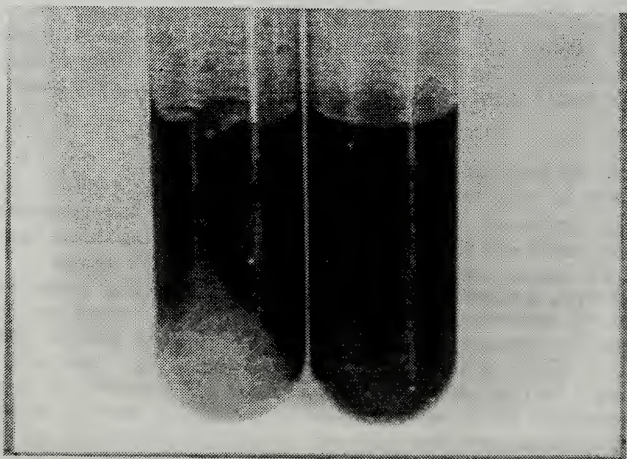


Figure 6. Centrifuge tubes containing starch in sugar solution. On the left, solution heated to 80°C for 1 hour producing gel precipitate. On the right, solution heated to 100°C for 1/2 hour producing solubilized starch.

In another centrifuge test series the gelatinizing process was studied. The gelatinization of starch proceeds gradually as the temperature is raised from ambient to 100°C. The granules of starch continue to swell and gradually become softer, but they do not become a uniform and homogeneous solution (even if maintained at 80°C for three hours), unless the temperature is maintained at 98-100°C for a minimum of one-half hour. If this minimum condition of 100°C and one-half hour is achieved, the gelatinized granules lose their integrity completely and form a solution. A different duration of heating may be required for different types of starch. In the solubilized form no precipitate deposit is obtained in the centrifuge.

The results of these centrifuge tests confirm previous observations (Bhangoo and Carpenter, 1966) that granules of starch sometimes appear in raw sugar.

The gel starch testing is extended to a set of conditions where at least 70% of the starch originally added to the sugar solution could be separated in the centrifuge. The resulting data were plotted (Figure 7). To provide usable data for planning a continuous centrifuge separation, the data were converted to g's versus the required duration. The equation used was

$$RCF = 11.17 r(RPM/1000)^2$$

where

RCF = relative centrifugal force (g)
r = radius (cm)

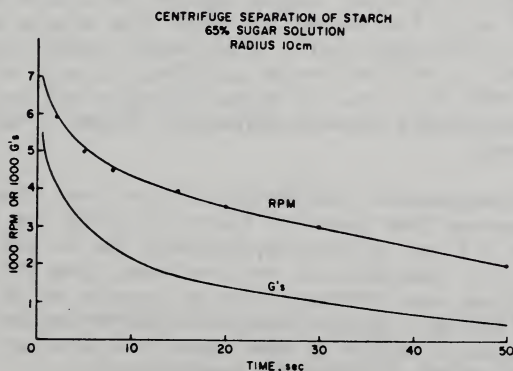


Figure 7. Centrifuge separation of starch 65% sugar solution radius 10 cm.

Based on design information on commercially available centrifuges, it was concluded that starch can be separated in a continuous centrifuge operation for separating indiginous starch in cane sugar. This method of starch separation in industrial scale has been considered uneconomical by the sugar industry.

FILTRATION

The filtration of sugar with minor constituents in it, such as starch and dextran, poses bascially two different problems. In the case of starch, the primary problem is that the solid granules cause the plugging of the filter and eventual stoppage of fluid permeation (Figure 8). However, when dextran is present the primary problem is the increased viscosity (resistance to fluid shear). Therefore, the test, analysis and evaluation of these two separate effects require parameters which provide meaningful evaluation of data.

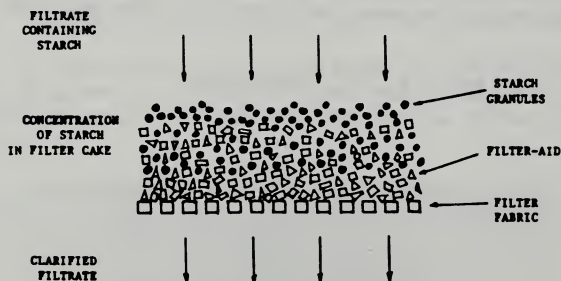


Figure 8. Permeability through filter and filter plugging by starch

Periodically measurements were made of filter cake thickness and it was observed that prior to filter plugging the filter cake thickness did not increase measurably due to the accumulation of starch. Therefore it was concluded that the rate of increase of filter cake thickness can be treated as an independent variable and as a simplification it can be ignored. For the determination of starch in the filter the mass per unit area was considered sufficient for analysis.

Three parameters have been selected: relative permeability, concentration of minor constituent in the filter cake and percent of minor constituent in syrup. These parameters are

readily obtainable in a laboratory, pilot plant or industrial scale refinery conditions. The relative permeability (dimensionless number) is

$$\text{Relative Permeability} = \frac{\text{Permeability of test solution}}{\text{Permeability of reference solution}}$$

Permeability has consistent dimension, such as ml/sec cm². The reference solution in laboratory tests was refined sugar (Table I).

Table I. Permeability Basis For Calculation

REFINED SUGAR SOLUTION 65% Filter-AID THICKNESS 1 cm PRESSURE:DIFFERENTIAL 200 kPa	
TEMPERATURE °C	PERMEABILITY ml / sec cm ²
80	0.269
70	0.209
60	0.156
50	0.0942
40	0.0434
25	0.0289

In a refinery the relative ratio of permeability of syrup containing abnormal constituent (e.g. high starch or dextran content) divided by permeability at nominal operating condition with good quality syrup. In filtration, permeability is the single most important parameter; it is simple to define and clearly visualized. Fluid permeation through porous media is a special condition where the flow is laminar and occurs when the Reynolds Number is less than 2000. The concentration of starch in the filter cake can be computed:

$$C_s = \frac{CVt}{A}$$

where

- C_s = specific concentration of starch in filter cake (e.g. g/cm²)
- C = concentration of starch in syrup (e.g. g/cm³)
- V = volumetric flowrate (e.g. ml/sec)
- t = time (sec)
- A = area of filter (e.g. cm²)

The assumption was made that all of the starch in the syrup was captured by the filter. This is a function of the type of filter used and when required, a factor may be used based on test data. The V_t term may be replaced by total volume of filtrate accumulated to a given time.

The two parameters, relative permeability and concentration of starch in filter cake were plotted to obtain a correlation. The advantage gained in this analysis is that a particular segment in the operating life time of a filter can be evaluated without resorting to the problem of determining when the mass transfer through the filter comes to a complete stop, which is a tedious task and irrelevant. This is particularly true in a refinery where the filtration is stopped and filters are cleared when the permeation drops to approximately 50%.

When a solution containing 1% starch is preheated at 80°C for one hour prior to filtration, a reversal in temperature is observed (Figure 9). That is, the relative permeability is increased by dropping the temperature from 80 to 70°C. These phenomena can be explained by the observation of the filter cake, where a powder type deposit of starch is noticed for temperatures 70°C and below, permitting a uniform permeation of starch in the filter aid. However, for 80°C, the starch granules have a more gelatinized adhesive characteristic and the permeation into the filter aid is not as uniform and clogs the filter sooner by accumulating more on the surface of the filter. With a further drop in temperature below 70°C, the exponential increase in viscosity is dominant and fluid permeation is obstructed by resistance to flow.

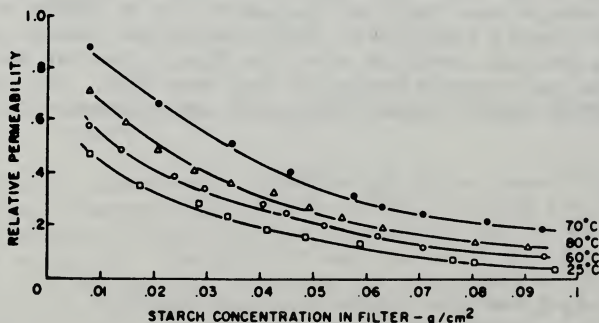


Figure 9. Relative permeability filter plugging by starch (65% sugar, 1% starch preheated at 80°C for 1 hr.)

The two equivalent filter aids used in this test program were Manville Celite 545 and Eagle-Picher Celatom FW-40. These filter aids were used in a vertical flow pressurized filter cylinder with a filtration surface area of 13.4 cm^2 . The permeability of syrup containing dextran is a function of the viscosity effect. At nominal operating conditions of 80°C the relative permeability dropped down to 0.8 for 1% dextran content. It dropped down to 0.5 for a 3% dextran content (Figure 10). This data confirms the magnitude of difficulty encountered by sugar refiners when a 3% dextran is encountered in raw sugar.

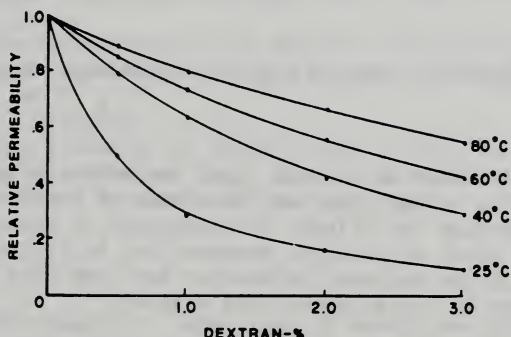


Figure 10. Relative permeability filter resistance by dextran

When both dextran and starch (gel granule) are present, a relatively low viscosity is observed as described above. Filtration tests comparing the three types of solution, i.e. 1% starch, 1% dextran and a mixture of 1% starch and 1% dextran, are compared on Figure 11 and confirm the viscosity measurements (Figure 3). In figure 10 the basis of reference for relative permeability is the 1% starch solution, which shows a decay of fluid permeation versus time as the starch gradually plugs the filter. A negligible filter plugging was observed for 1% dextran and the lower fluid permeation was due to a higher viscosity. When both starch and dextran were present, initially the permeability was higher but gradually decayed due to filter plugging by the starch granules.

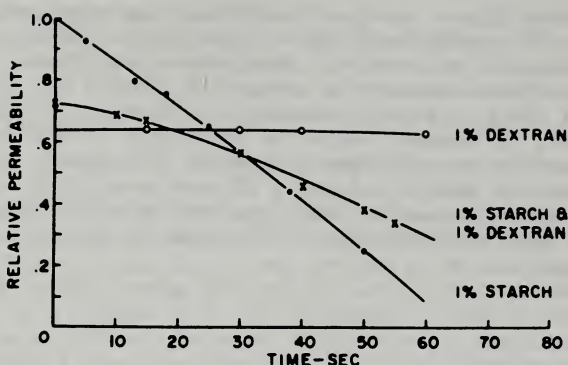


Figure 11. Relative permeability 70°C, 65% sugar solution

CONCLUSION

The presence of dextran in cane sugar can double or triple the viscosity value of the syrup and thus make it harder to pump the liquid through the filters in proportion to viscosity increase. A study of relative permeability in filtration indicated that the refinery production rate can drop to 50% when raw sugar is contaminated with dextran.

The presence of starch in raw sugar contributed to both increase in particulate matter and viscosity, the latter effect resulted in decrease in permeability. The testing in this series was conducted with corn starch, which closely resembles sugar cane starch. The variety of indigenous starch present in sugar cane is dependent upon the cane variety and the crop environment. The ordinary refinery conditions are 80°C, however, in the clarifiers and evaporators the temperatures can reach 100°C, which can solubilize the starch. The 80°C temperature condition alone would not solubilize the starch as determined by the above described tests. The presence of gel starch granules in raw sugar remains controversial in view of the frequent comment made by local refineries that whenever measured starch content exceeds 0.002% (2 ppm) filter plugging problems are encountered.

It was observed that presence of gel starch even at relatively high percentages of 1% weight of sugar did not increase the filter cake thickness noticeably. That is, the gelatinized granules permeated into the filter aid and plugged the filter sufficiently to make it necessary to stop the filtration and to clean the filters prior to a measureable increase in filter cake thickness had accumulated due to starch.

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DISCUSSION

J. B. ALEXANDER (Hulett's): What type of starch was used in these tests? Was it in a granular form? Do you think that the starch that was used was representative of the starch found in raw sugar?

G. M. ELGAL: We used corn starch which is the closest in resemblance to indigenous starch occurring in sugarcane. The particle size was 5 to 30 microns which is typical of sugarcane starch. The problem is to define exactly what is native or indigenous starch in sugarcane. I would expect starch from different varieties or from different geographical areas would be slightly different.

J. B. ALEXANDER: Do you think that the form in which you used your starch was representative of the starch occurring in raw sugar. We believe that by the time you get to raw sugar the starch that is co-crystallized or has been included in the crystal is no longer in a granular form at all, and that by using granular starch you could well be misrepresenting the position.

G. M. ELGAL: I have read articles along those lines, and there is a problem in semantics. The inference quite often is that by heating to 70° or 80°C, the starch is solubilized, but as we saw in the centrifuge tests, strictly speaking it is a granular gel, not solubilized. It is not in the form of a homogeneous

mixture, or a solution. Also, in the gel form, starch can be attacked by enzymes. It does not need to be solubilized.

J. B. ALEXANDER: In our manufacturing process, the starch is heated to more than 100°C so we have put that granular starch into solution. By the time it has gone through the syrup stage and crystallization it is certainly no longer in the granular form.

G. M. ELGAL: The question that has been on my mind is how long is the sugar solution heated to 100°C . I get the impression that even in the factories, the solution is not kept more than 1/2 hour at a temperature near 100°C . If it is not heated longer than this, I am convinced that the starch could still be in granular form or gel form.

F. G. CARPENTER: Some 18 years ago in a clarifier study we were examining many raw sugars with a microscope. At that time, I too felt that any starch in raw sugar would surely be solubilized. Apparently there are at least some cane starches that are not solubilized by the usual heating received in a raw house. Under the polarizing microscope the starch stands out very unmistakably and we saw a lot of starch granules in a lot of raw sugars that just should not be there.

G. VANE (Tate and Lyle): Did you do any measurements at all where you had both dextran and starch together?

G. M. ELGAL: Yes, we did but the data obtained were somewhat inconsistent so we must go back and check that. The viscosities were not additive. We will have to check this again.

COLOR AND HYDROXYMETHYL FURFURAL IN RAW SUGAR

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INTRODUCTION

The problems of storage of raw sugars and deterioration in quality are generally well known and have been studied by several groups of workers. Carpenter and Petri (1979) reviewed the problems and compiled relationships between color gain, pol loss, and storage time. South African technologists have examined these problems (Kimmerling, 1975) and Russian and Cuban workers have published many papers in this area (Muro et al, 1974, 1976; Durak, 1976; Zaborsin et al, 1977). An intensive study of color development on storage was made in the Philippines (Samaniego and Salaiman, 1974).

Increasing temperature and relative humidity are the major adverse effects on quality. The pH generally goes down, because of the decomposition of invert to color and organic acids. The effect of polysaccharide levels on storage conditions has not been observed.

There are two different sets of conditions relevant to storage changes in raw sugar in piles: conditions occurring in the outer coating of the pile, for the first 6 to 8 inches depth of sugar, and conditions occurring with the pile, where the sugar is insulated to a great extent from temperature and relative humidity changes on the outside.

The goal of this study is to examine changes under conditions inside the sugar pile. Conditions that are recognized as the outside acceptable limits for storage i.e. 100°F, were chosen. Samples were in closed containers with no continuing supply of air. Relative humidity was not considered. These conditions simulate general limiting storage conditions, so that deterioration observed would be less severe than at higher temperatures and free access to ambient air. The intention of the study was to simulate general practical conditions and to observe what deterioration might then be expected.

This storage was begun in 1979, and this paper is a preliminary report on findings in changes in color, sucrose, glucose and fructose content of the stored sugars. Individual colorants and precursors, and colorant profiles, will be included in the study, in an attempt to find compounds that indicate potential behaviour under storage conditions. It had been postulated (Clarke, 1980) that 5-hydroxymethyl-2-furfural (HMF), an acid degradation product of sucrose via fructose, could be such an indicator. HMF is not colored itself, but quickly degrades to form dark colored compounds.

EXPERIMENTAL

Materials

Raw sugars were selected from the following countries of origin, to have a variety of characteristics:

The Philippines (a very dark sugar stored for several years)

Dominican Republic (an average raw sugar with no apparent special problems)

Natal (a high quality raw)

In addition, a high grade first strike refined sugar was selected for use as a reference. The sugars were stored in 8-ounce screw top glass jars, filled, in an oven held at 37.8°C (100°). Samples were removed at intervals of a few months (sufficient samples were prepared to allow for several years storage) and stored in a freezer at -8°C.

Methods

Polarizations were performed on a Perkin-Elmer 241 MC automatic polarimeter, thermostatted and water-cooled at 20°C. Sugar solution colors were measured on a Talameter at 420 nm. Solutions were filtered, and adjusted to pH 7 on an Orion Model 701 pH meter. Brix were read on an Abbe' refractometer.

Sugar analyses (sucrose, glucose and fructose) were performed by HPLC on a Waters Associates Model No. ALC/GPC 244 chromatograph, with Model 6000A pump, R-40 differential refractometer and Model 440 absorbance detector, with 254 nm filter, and a Waters Associates WISP 7108 automatic injection system. Chromatograms were shown on a Linear 505 recorder. A Hewlett-Packard laboratory automation system to which the chromatograph is connected, was used to process the data. The column was BioRad HPX-87C resin, at 88°C, with 20 mg/litre calcium acetate solution as eluent. Analyses for hydroxymethylfurfural were made by the spectrophotometric method of White

(White, 1979), using a Beckman DB spectrophotometer for readings. Calibration for sugars is accomplished by injection of solutions of standards of glucose, fructose and sucrose at varying concentrations. Standards that are appropriate for the amount of sugar under test in the unknown sample are selected (peak area in the parameter measured) and calibration charts, of short range to be as linear as possible, are chosen for the appropriate samples. An example is shown in Figure 1, where fructose standards in the range of 5 to 20 μ g, are calibrated. The correlation coefficient for this calibrated curve is 0.9997.

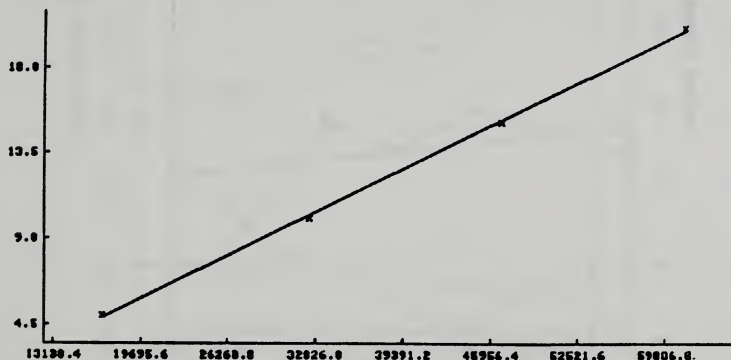


Figure 1.--Fructose standards

Sugars were washed by the Hawaiian method (Meade-Chen, 1977) using first several portion of 66 Brix white sugar syrup, removed by vacuum, then methanol, and air drying.

Results and Discussion

The color change of the refined white sugar, shown in Figure 2, and Table 1, is essentially zero. The small changes observed and the overall drop in color are no doubt caused by sampling errors. This was a very high quality refined 1st strike, chosen for that reason so that changes exhibited on storage would be caused by sucrose alone, and as little as possible by non-sucrose components.

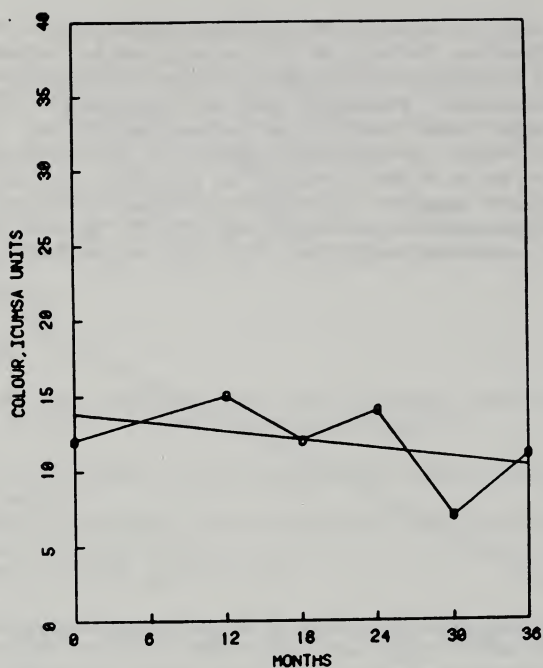


Figure 2.--Refined white sugar

Table 1.--Color, high grade refined, ICUMSA units

Time (months)	Refined sugar
0	12
12	15
18	12
24	14
30	7
36	11
mean = 11.8	
C.V. = 23.6%	

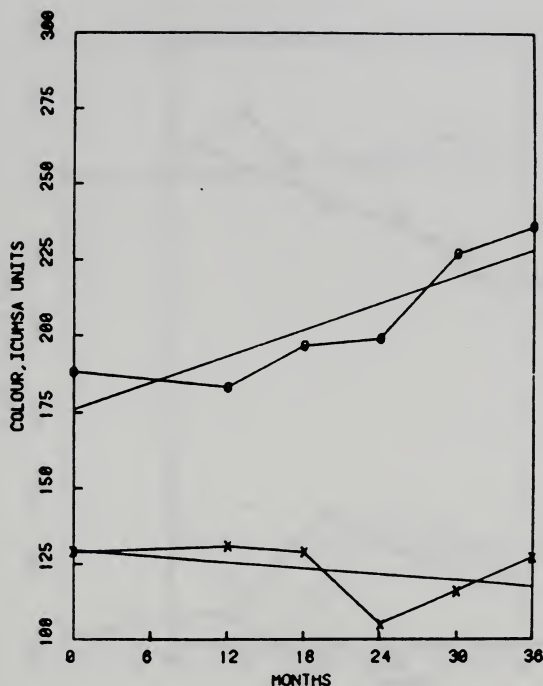


Figure 3--Natal Raw

Color changes in the raw sugars are shown in Figures 3, 4, and 5, and Tables 2, 3, and 4. The Natal raw was chosen as an example of a good raw; the Dominican as an average, and the Philippine as a poor, very dark raw with small grain, and lumps of molasses. The general rule is that the poorer the raw, the greater deterioration occurs on storage. In this case, however, the average (D.R.) raw showed much greater color development, (rate of color development is the slope of the curve of color v/s time) than the poor raw, with a slope

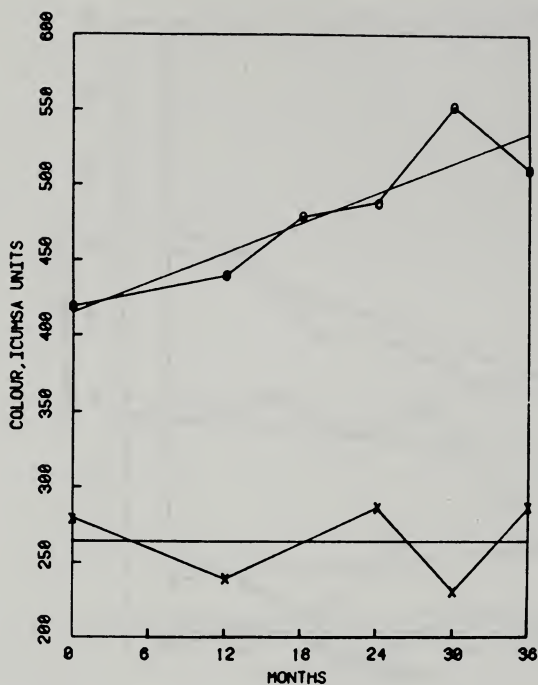


Figure 4--Dominican Republic Raw

Table 2.--Color, Natal raw, ICUMSA units

Time (months)	Raw	Washed raw
0	188	129
12	183	131
18	197	129
24	199	105
30	227	116
36	236	127
$r = 0.87$ slope = 1.46		mean = 123 C.V. = 8.3%

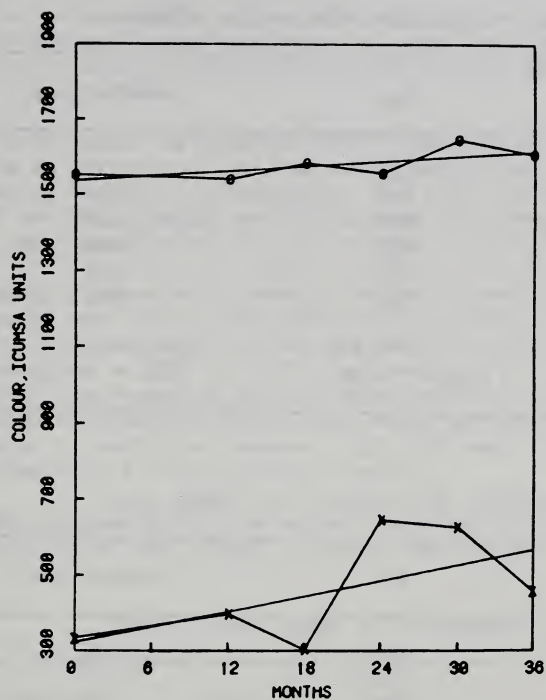


Figure 5-- Philippines Raw

Table 3.--Color, Dominican Republic raw, ICUMSA units

Time (months)	Raw	Washed raw
0	419	279
12	440	239
18	479	(133)
24	488	286
30	552	230
36	510	286
	$r = 0.89$	mean = 264
	slope = 3.30	C.V. = 10.3%

Table 4.--Color, Philippine raw, ICUMSA units

Time (months)	Raw	Washed raw
0	1551	334
12	1540	397
18	1583	303
24	1556	644
30	1644	623
36	1606	457
	$r = 0.72$	mean = 460
	slope = 2.2	C.V. = 31.5%

of 3.3 for the D. R. raw, and 2.2 for the Philippines. The good raw showed a slope of 1.46. Correlation coefficients for color development with time are not especially high.

The washed sugars, which were probably overwashed by refinery standards as laboratory washed sugars usually are, showed very little color change. The Natal color appears to have decreased, but this must be an artifact from sampling error or overwashing.

Data on washed sugars, shown in Tables 2, 3, and 4, are therefore treated as linear data. The coefficients of variation, of 8.3% and 10.3% for the Natal and D.R. respectively, support this treatment. The data on the Philippine raw are more scattered, and show an increase in color too large to be

attributed to sampling error. The high percentage of color and non-sucrose components here must mean that color can continue to develop within the crystal in very highly colored, poorer quality, raws.

Color development is known to occur primarily in the syrup coating on the crystal, so that color development in storage is usually removed by affination. The color in the crystal itself has been thought not to increase in storage but apparently can sometimes build up in especially poor sugars.

In comparing these color developments with predicted figures (Carpenter and Petri, 1979) (Muro et al, 1976), color development is less than expected for the Natal (predicted: 50% increase/year at 86°C and 100% increase/year at 113°F), and much less than expected for the lower quality raws. It must be remembered that storage in this study was under center-of-the-pile conditions, however, and that much greater color development will occur in outer layers.

The HMF analyses on the raw sugars are shown in Table 5. HMF analyses can be run by HPLC (Clarke, 1980) on an ethyl acetate

Table 5.--HMF (ppm) of raw sugars

Time (months)	Natal	D.R.	Philippine
0	55	70	172
12	64	71	119
18	64	76	64
24	65	43	70
30	64	65	68
36	113	70	30
Slope	8.31	-1.46	-24.5

extract of the sugar, using a reverse phase column. A simpler analysis had been achieved on an ion-exchange carbohydrate column with U-V detection at 254 nm, but this proved difficult to reproduce with subsequent columns. The spectrophotometric analysis was intended for use as a reference method for the HPLC determination. However, results showed no particular correlation of HMF appearance or disappearance with color, or time of storage, and there appeared little reason to pursue further methods of analysis. Table 6 shows the relationships between HMF content and the colors of the raw sugars. The only interesting observation is that the Philippine raw, which

Table 6.--Color and HMF

	Natal		D.R.		Philippine	
	ICUMSA	ppm HMF	ICUMSA	ppm HMF	ICUMSA	ppm HMF
	188	55	419	70	1551	172
	183	64	440	71	1540	119
	197	64	479	76	1583	64
	199	65	488	43	1586	67
	227	64	552	65	1644	68
	236	113	510	70	1606	30
Slope	0.72		-0.51			-0.93
r	0.74		-0.21			-0.70

developed color in the crystal as well as in the coating, did have a high initial HMF content, supporting the original postulate that HMF might be used as an indicator of potential color development.

Tables 7 and 8 record changes in polarization. The Dominican raw, which had an average initial color of 419 ICUMSA units, had an initial pol of only 96.08, whereas the Philippine, of higher color, was actually of higher pol. This may account for the greater than expected decrease in pol and increase in color formation in this D.R. raw compared to the Philippines sugar. The figures on the Natal in Table 8 compare with Carpenter's estimate (Carpenter and Petri, 1979) of about 0.2% pol loss per year for a good raw sugar stored at 86°F. Pol

Table 7.--Change in pol

Time (months)	D.R. raw	Philippines raw
0	96.8	98.78
12	95.66	96.26
18	94.52	95.36
24	94.98	95.83
30	93.34	94.75
36	93.28	94.31
Rate of pol loss/6 months	0.50	0.69
r	-0.02	-0.94

Table 8.--Change in pol

Time (months)	Natal raw	Refined white
0	98.12	99.85
12	97.94	99.70
18	97.68	99.63
24	97.75	99.60
30	97.63	99.60
36	97.46	99.42
Rate of pol loss/6 months	0.10	0.08
r	-0.96	-0.96

losses on the D.R. and Philippines are in the same area as others in the literature (Muro et al, 1974) (Priester, 1976), indicating that pol change under centre-of-the-pile conditions may be more severe than color, compared to changes in the outer layers.

High pressure liquid chromatographic results are shown in Tables 9, 10, and 11. Sucrose is represented as percent of the original sucrose in the sugar. The sucrose value after storage, if compared to pol value, reads lower than the pol value. The Philippines pol value, after 36 months storage, is 95.47, and the Natal, 99.3% of original pol. There appears to be greater loss in sucrose than in pol. HPLC values for sucrose are generally lower than pol values (Clarke and Brannan, 1978; Damon and Pettitt, 1980), in part because of

Table 9.--Change in % of sucrose by HPLC

	Philippine Raw	Natal raw
0	100	100
10	96.28	99.80
18	94.97	99.80
24	94.32	(103.5)
30	-	98.2
36	94.69	97.71
Rate of sucrose loss/6 months	0.894	0.4110
r	-0.06	-0.92

Table 10. % Glucose and fructose by HPLC

Time	D.R.		Philippine	
	Glucose	Fructose	Glucose	Fructose
0	0.39	0.18	0.57	0.46
12	1.12	0.81	0.75	0.80
18	0.37	0.31	0.46	0.42
24	0.34	0.28	0.49	0.43
30	-	-	0.48	0.95
36	0.62	0.55	1.27	0.79

Table 11.--% Glucose and fructose by HPLC

Time	Natal		Refined white	
	Glucose	Fructose	Glucose	Fructose
0	-	0.02	-	-
12	-	0.04	-	-
18	0.42	0.26	-	-
24	-	0.05	-	-
30	0.16	0.02	-	-
36	-	-	-	-
Limit of detection	0.008	0.008	-	-

the effect of non-sucrose components on pol. Tables 10 and 11 show the changes in concentrations of glucose and fructose in the sugars under storage. No definite trend is observable: there is continual formation of glucose and fructose from inversion, and decomposition of these sugars into colored compounds, primarily anionic in nature. Glucose and fructose were undetectable in the white sugar i.e. present at levels below the limit of detection of this system at 0.008%.

SUMMARY

This preliminary report on the sugar storage study has examined raw sugars of varying qualities and a refined sugar stored up to 36 months at 100°F for changes in color, pol, hydroxymethylfurfural content and sucrose, glucose and fructose content. Results indicate that color formation occurs in the syrup layer coating of the crystal, except in the case of a

very highly colored raw, where color in crystal also increases. HMF does not appear to correlate with color formation although it may be an indicator of subsequent color development. Sucrose loss was slightly lower than pol loss.

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DISCUSSION

P. SMITH: Can you define more clearly what you mean by precursors?

M. A. CLARKE: We are talking about a class of compounds, or classes, not necessarily related chemically, that are not colored themselves but are known to form color, that is to react to form colored compounds, in sugar processing at some stage. Ferulic acid is an example; vanillin is another; when either of these is heated in sugar, colored compounds are formed.

P. SMITH: Did you measure, or do you intend to measure organic nitrogen, and amino nitrogen. I believe that this reaction is like the Maillard reaction. We did this many years ago in Australia before we had model systems. The coloured product seemed to act like le Chatelier's principle; when it started building up it slowed up the reaction. As soon as you deliberately took away the coloured product, the reaction speeded up again. It follows close to that observation on a very dark Philippine sugar.

M. A. CLARKE: By what means did you take the reaction product away?

P. SMITH: We decolourized the final dark sugar solution, and then reheated it once more.

M. A. CLARKE: Yes, I am sure that there are many Maillard reaction products involved here and measurement of both amino and non-amino nitrogen is included in our plans.

M. C. BENNETT (Tate and Lyle): You have many sugar samples in storage and I understand that you do not have a complete history of how they were made in all cases. I wonder if you could do a sulfite analysis on some of the sugars. There should not be sulfite in South African sugar but there may be some in Dominican Republic sugar. I think that it would be interesting for many people to see the sulfite analysis, since sulfur dioxide is blamed as one of the culprits for instability. Would you like to comment on that?

M. A. CLARKE: We don't have the history of all the sugars, and in history is included how they were boiled; e.g. Einwurf or double magma or whatever system. This knowledge would tell us whether the crystal is homogeneous or in layers.

We will do sulfite analyses on the sugars: sulfite analysis and sulfite reactions are on our research plan for future work. Sulfite is coming more and more into use around the world in new processes - not the old sulfitation towers that everybody in the tropics has always used, but in new juice sulfitation

processes. I think that it is important that we find out more about what reactions go on with SO_2 in sugars. Our goal is to find out the probable behavior of sugars containing sulfite reaction products in refineries, or in storage, or for sugar users.

C. J. NOVOTNY (Industrial Filters): How did you wash the raw sugars?

M. A. CLARKE: We used the Hawaiian system. Those washed raws were probably over-washed because laboratory washing is usually over-washing. The Hawaiian system uses 4 applications of a 60 Brix white sugar syrup, filtered off with a vacuum, and subsequent washing with isopropanol and methanol. (Cane Sugar Handbook, 10ed, p. 682.)

J. ALEXANDER (Hulett's): That was a very interesting paper. In regard to the history of the samples, you mentioned the Natal one as being VHP. We would not class it as such. We would call it a coated sugar. I would be interested to know where you got it, because from its polarization I would judge it to be between a Japanese assortment of sugar and the normal high pol sugar which is about 98.7. It seems to be a very strange animal.

M. A. CLARKE: I will look up where we got it and tell you after the session.

EFFECTS OF DEXTRAN ON SUCROSE CRYSTAL SHAPE

Michael Saska and J. A. Polack

Louisiana State University

The problems allegedly caused by dextrans in sugar production are well-known and well-reported. As far back as the 1930's, the occurrence of needle grain in boiling juice from frozen cane was ascribed to dextran, formed by *Leuconostoc Mesenteroides*. We are concerned with the nature of the action of dextran. What is the mechanism of needle grain formation? The following facts seem to be incontrovertible:

1. The addition of dextran to pure sucrose solutions causes only mild elongation.
2. The addition of dextran to impure solutions (e.g. molasses) yields needle grains.
3. Treating molasses which form needle grains with dextranase eliminates or minimizes the elongation.

Our conclusion from these facts is that the occurrence of needle grain is the result of synergism between dextran and another substance or substances present in commercial liquors. In the absence of dextran, marked c-b elongation does not occur. With dextran alone, the amount of elongation is limited, but with dextran and other materials present, needle grains inevitably are formed.

The present work is part of a longer range study the goal of which is to establish the mechanism of this action. In this report, we present the results of laboratory boiling studies in which the influence of several materials on observed elongation was measured. The boilings were carried out in a laboratory pan similar to one reported on at the last SPRI meeting (Devereux, 1980). It is a pyrex flask, equipped with stirrer, thermometers, and vacuum take-off line. The flask is surrounded by a water jacket, held at constant temperature. When appropriate supersaturation is reached, vacuum is momentarily broken, a batch of uniform seed crystals is added, and boiling conditions are quickly reestablished. Syrup feed is then initiated, simulating a commercial pan operation. At

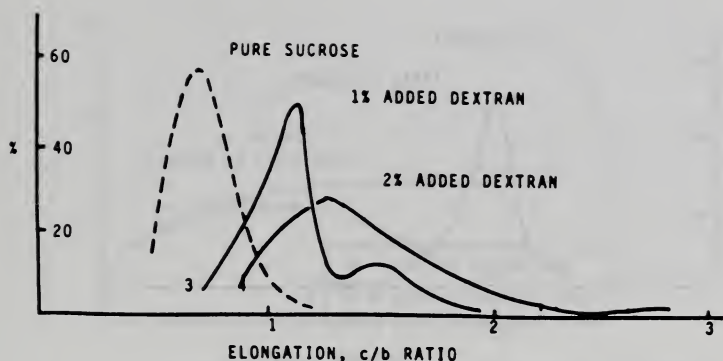


Figure 1.--Crystal elongation distribution.

the end of the boiling, the massecuite is sampled under vigorous stirring, and the sample is spread on a glass plate and photographed promptly. On the prints, fifty randomly selected crystals were measured along the b and c-axes. Only those crystals were considered the crystallographic structure of which could be positively identified. This is very important in samples of high variance.

The results were expressed in the form of frequency versus c-b elongation plots. Examples are shown in Figures 1 and 2. In the first of these, the results for sucrose are shown, with a mean elongation of about 0.75, which agrees well with the literature values. The addition of 1 and 2% commercial dextran T40 (M.W.=40,000) showed elongation to 1.1 and 1.5 respectively.

When a final molasses (prepared by dissolving sucrose in 38 purity molasses at high temperature to bring the final purity to about 67%) is the mother liquor the elongation is about 1.2, shown in Figure 2. Addition of 1% dextran T40 to this medium immediately gives a big spread in c-b elongation and a high average value, above 2.

In addition to dextrans of several molecular weights and concentrations, we looked at the addition of fructose, invert sugar, and oligosaccharides as produced by partially hydrolyzing dextrans. The theory in the latter case is that these lower molecular weight oligomers must be present in naturally occurring dextran solutions as precursors to the ultimate polymers. The Sugar Milling Research Institute of South Africa (1977), first suspected and later disproved kestoses as the culprit, and Kelly (1981) has proposed 1-6 isomaltose, coming from dextran hydrolysis as the responsible species. What we did was to partially hydrolyze both T10 (M.W.=10,000) and T40 dextrans in hydrochloric acid and

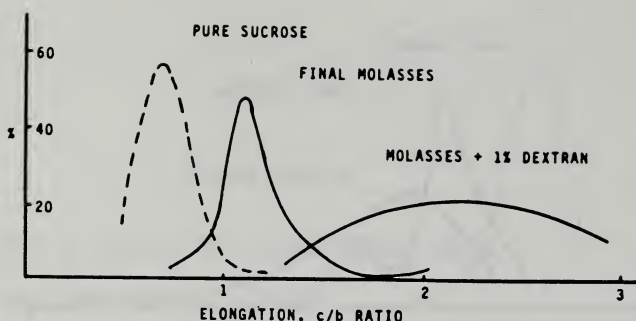


Figure 2.--Crystal elongation distribution.

established that hydrolysis had in fact occurred by virtue of the decrease in specific rotation of the resulting material.

The results of all of these tests are summarized in Table 1.

Several things are evident from these results. First of all, the experimental technique was verified by the results on pure sucrose and on sucrose with the addition of 2½% raffinose. These results are in close agreement with those in the literature. Second, the addition of dextran to the pure sucrose solutions caused some degree of elongation, as expected. The partially hydrolyzed dextrans gave results that were almost identical to those with the unhydrolyzed dextrans, if anything causing less elongation.

Table 1. Experimental Results

Sample or Additive	Mean Elongation	δ
Sucrose	0.75	0.2
2.5% Raffinose	0.38	0.14
0.3% Dextran T10	0.90	0.29
0.3% Dextran T40	1.13	0.42
1% Dextran T40	1.10	0.27
2% Dextran T40	1.53	0.74
0.3% Dextran, partially hydrolyzed	0.72	0.10
0.7% Dextran, partially hydrolyzed	1.15	0.33
2% T40 + 5% Fructose	1.38	0.62
1.9% T2000 + 10% Invert	1.60	0.66
Final Molasses	1.18	0.31
Final Molasses + 1% T40	2.45	0.91

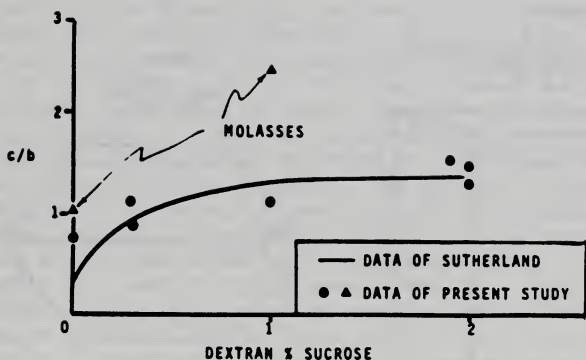


Figure 3.

None of the synthetic solutions matched the elongation occurring in molasses to which dextrans have been added. Thus, we have not yet succeeded in duplicating commercial needle grain. The closest approaches came from 2% dextran with or without the addition of invert sugars. These are pretty high levels of dextran addition.

One interesting trend in these data is that the higher the elongation the greater the standard deviation of the measurements. This means that the greater the elongation, the more spread there is among particles of low and high elongation. This is well illustrated by the flat spread out curve for the molasses with added dextran. In this system, there are all kinds of particles there, some lightly elongated and others very needle-like. The data on other systems show substantially less variance. The most precise of all are the runs with added raffinose, where the elongation is along the b-axis and is quite specific and reproducible. The generally high variability of the results makes statistical treatment mandatory.

The results are consistent with previous findings as well evidenced by Figure 3, which shows elongation as a function of percent dextran. The line shown is that of Sutherland (1969). The present data are in excellent agreement with the line. We are left with these two high points, one, with dextran-free molasses, and the other for molasses with added dextran. One must conclude that we have not yet found the culprit combination leading to needle grain.

This work was done by Mr. Saska in partial fulfillment of the requirements of the Master's Degree in Chemical Engineering at

FIGURE 4

EXAMPLE OF COMPUTER GENERATED
SUCROSE CRYSTALS
 $c/b = 1.10$

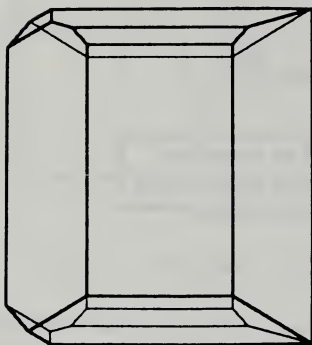


FIGURE 5

SAME CRYSTAL
TOP VIEW

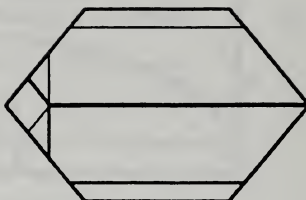
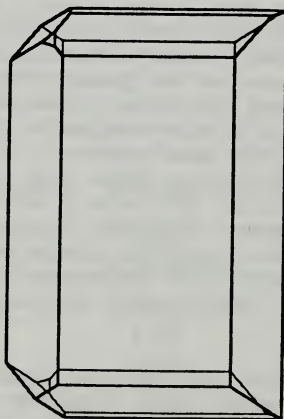


FIGURE 6

MODERATELY ELONGATED
CRYSTAL (AFTER KELLY)
 $c/b = 1.49$



Louisiana State University. We hope that future graduate students will continue this work. If we can find the specific materials responsible for elongation, perhaps we can find a new way of dealing with it. Mr. Saska himself is continuing this work, approaching it from a theoretical rather than empirical point of view. It is possible to calculate a theoretical crystal habit provided one has enough information about crystal surface energies. The sucrose crystal can be simulated, on a computer, starting with established information about interatomic distances and assuming that forces between the atoms can be approximated by a potential function with empirically chosen parameters. Foreign bodies adsorbed specifically on given surfaces modify the surface energies and hence the ultimate shape of the crystal. Examples of computer-grown sucrose crystals are shown in the next Figures 4, 5, and 6. Figure 4 is an average cane sugar crystal as measured by Kelly, et al (1981). It has a slight c/b elongation of 1.10. Figure 5 is a top view of the same crystal; it shows the power of the computer in the hands of a knowledgeable operator. Figure 6 shows a typical elongated crystal, again according to Kelly, with an elongation of 1.49.

It is to be hoped that both theoretical and empirical approaches will eventually lead to an understanding of the causes and cures of needle-grain formation.

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DISCUSSION

F. W. PARRISH: At what point does the c/b ratio become a problem?

J. A. POLACK: I would like to refer that question to the refiners. As far as the raw sugar manufacturer is concerned, I don't think that there is a known dimension. When they get a problem with needle grain, they get it all of a sudden. It is excessive. It is not a gradual increase.

T. BALOGH (Redpath): We don't like to get needle grain sugars at all. If we see needle grain, we know that there was a problem prior to us receiving the sugar.

M. A. CLARKE: How is the crystal elongation measured?

J. A. POLACK: Mechanically, pictures were taken. Having that fixed then on paper, about 50 crystals that could be identified as to axis were measured.

M. A. CLARKE: In the crystallization of molasses with added dextran, did you add sucrose to the molasses to crystallize it out, or did you use a high purity molasses?

J. A. POLACK: It was in a regular laboratory boiling with the addition of a seed. We added sucrose to form a seed and allowed it to grow, and these crystals elongated. I don't know whether it should be called needle grain when the average ratio was 2.5.

M. SASKA (Georgia Inst. Technology): The definition of a needle is very subjective. It did not look as pronounced as some industrial cases. It was elongated; there is no doubt about it. In some severe cases that ratio may be up to 5 or 6.

M. A. CLARKE: Referring back to the curve that you showed on Sutherland's data, which your own data fitted very well: Fred Parrish and I have commented that in that data of Sutherland, he starts with a c/b ratio below 1.0, in fact of 0.5, which is not elongation. You have just said that a ratio of 5 is not uncommon. Needle grain could not be classified as such until the axis ratio of c/b is greater than 1, yet about half of that graph shows c/b less than 1. Would you like to comment on this?

M. SASKA: I think that we are interested in change of shape in general in this direction. This ratio for pure sucrose is about 0.7; so every thing over that can be considered elongation. It is our assumption that the cause behind the habit modification of different extents is the same from only slightly elongated crystals (approx. 1) up to extreme cases of needle grain (6 or more).

J. A. POLACK: I think it might be added that raw cane sugar, the normal sugar, has a c/b ratio of about 1.1. This ratio for pure sugar is 0.75.

C. C. CHOU: I have comments about the effect of particle shape and size on refinery problems. First, it is very difficult to spin because the compaction is different when you have needle shape. Second, the elongated particles tend to go through the screen on the c axis. This causes problem in the PD purity. Of course there are others. I have a question. What is the effect of the particle size on the c/b ratio. It has been my impression that, other things being equal, for 50 microns versus 200 microns, the c/b ratio is different. This has been observed from time to time.

J. A. POLACK: I don't believe that we have data on that, so, I would just have to give an opinion. It seems to me that when you have conditions that give you trouble with needle grain in the raw factory, you have trouble nucleating in the first place, and when you see the needles you have billions of fine particles. In these experiments we were starting with a uniform seed that was larger in size to begin with. The elongation occurred from crystals that were not originally elongated, but under the growth conditions did become elongated.

C. C. CHOU: I would like to see some data on c/b ratio at about 10 micron, 50 micron, 200 micron, respectively. In a refinery remelt pan you can really see the effect of size on the elongation. Would you care to speculate on the mechanism and kinetics for elongation?

J. A. POLACK: Speculation, sure. It is quite easy to show that if an impurity is adsorbed on the surface of a crystal, it will impact on the surface energies, or on the growth rates of that face. If you merely assume that changes in those properties take place, then the model results in an elongated shape. It is adsorption of certain species on certain faces that modify the surface diffusion or the surface energies. This is not very helpful because you can't go out into the plant and do any thing about that.

M. C. BENNETT (Tate and Lyle): I think that the last question and answer come down to the root of the whole problem; what causes it and how does it cause it. Dr. Chou asked what Dr. Polack's opinion was and Dr. Polack rightly said you have got adsorption on a face. In fact we know that it is not adsorption on the face of a crystal, but rather on the step on a face of a crystal. So, we are in three dimensions, not two. We also know that the adsorption has a "lock and key" mechanism. What we are looking for is something that slots precisely into a step which is perhaps 50 or 100 molecules deep. It is the step that rolls across the face which causes

the crystal to grow. We are looking for something that gets right in at the angle of the step, something that just clicks into there and stops that step rushing across the face of the crystal. If we could find out what that is, we would probably know then what to do about it. Almost certainly, if you have a change of crystal shape, you will have a change of crystallisation rates and that is of equal commercial importance.

J. REID (Atlantic): In Figure 1 there was an interesting little hump on the 1% dextran added. Would you care to comment on that?

M. SASKA: We observed several of those peaks on several samples and what we observed in those samples was that the small crystals were more elongated than the large ones. This is in agreement with the remarks of Dr. Chou. We suspect that it is because the small crystals may have been nucleated instead of growing from a seed. This may be the effect of initial seed size or initial seed shape. Those that are nucleated start from zero initial size and the effect of initial size and shape is minimal.

A QUANTITATIVE METHOD FOR DEXTRAN ANALYSIS

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Sugar Processing Research, Inc.

INTRODUCTION

Dextran is an α -1,6 linked glucose polymer with random α -1,3 and α -1,4 branching. It is produced from sucrose by a large number of microorganisms (Jeanes et al 1977). The most prevalent of these is Leuconostoc mesenteroides which is generally the source of the dextran in sugarcane products. The presence of dextran in sugar represents not only a loss of sucrose but an increase in substances which cause problems in the manufacturing and refining processes. Imrie and Tilbury (1972) published an excellent review of dextran formation and the problems it causes in process.

A method was devised by Nicholson and Horsley (1959) for the determination of dextran and revised by Hidi et al (1976). This method is commonly known as the alcohol haze method, or CSR method (Meade Chen 1977). This is, so far, the only method suitable for practical factory control analysis but it has a number of shortcomings. It is not specific for dextran and the results obtained are dependent upon the molecular weight of the dextran in the sample, and the molecular weight of the dextran used as standard.

This report describes a new quantitative method for dextran in which all the polysaccharides are separated from the sugar and the dextran is selectively precipitated with alkaline copper sulfate (Hint and Thorsen 1947). The dextran in the precipitate is then determined colorimetrically (Dubois et al 1956). Neither starch nor the indigenous sugarcane polysaccharide (ISP) are precipitated by copper sulfate and therefore do not interfere in the method. Protein and salt do not react with the colorimetric reagents. The method is fairly rapid, the results are reproducible and independent of the molecular weight of the dextran. Because the dextran is separated out from the sugar sample, this test is suitable for use on dark colored liquors and syrups.

EXPERIMENTAL

Reagents

Absolute ethyl alcohol.

80% Alcohol. Dilute 80 ml of absolute ethanol with 20 ml of distilled water.

2.5N sodium hydroxide solution. Dissolve 100 g of sodium hydroxide in water, dilute to 1000 ml and saturate with sodium sulfate. Store in a reagent bottle with stopper of material other than ground glass.

Copper reagent. Prepare a stock solution by dissolving 3.0 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 30.0 g of sodium citrate separately in small amounts of water, then mix the two and dilute to 1000 ml with distilled water. To prepare the reagent, dilute 50 ml of the stock solution with 50 ml of water and dissolve in this 12.5 g of anhydrous sodium sulfate. This reagent must be freshly prepared each day.

5% Phenol solution. Dissolve 5.0 g of pure phenol in water and dilute to 100 ml.

Wash solution. To 50 ml of water add 10 ml of the copper sulfate reagent and 10 ml of 2.5 N sodium hydroxide.

10.0% Trichloroacetic acid solution. Dissolve 10.0 g of trichloroacetic acid in water and dilute to 100 ml.

Concentrated sulfuric acid.

2.0N Sulfuric Acid. Dissolve 98 g of concentrated sulfuric acid in water and dilute to 1000 ml.

PREPARATION OF STANDARD CURVE

Determination of moisture content of dextran: Weigh 500 mg of dextran T2000 or other standard dextran in a weighing dish and dry in an oven at 105°C for 4 hrs. Weigh and calculate the moisture content by difference. For example: weigh out 1.9418 g of dextran T2000 in a tared dish and heat in an oven at 110°C for 4 hrs. Allow it to cool in a dessicator over anhydrous calcium chloride. Weigh again and subtract the weight of the dish. The dry dextran weighs 1.7710 g.

$$\text{Then } \frac{1.9418 - 1.7710}{1.9418} \times 100 = 8.79\%$$

moisture. Then to weigh out 500 mg of dextran less the moisture content, weigh 500 mg + 8.79% of 500 mg = 543.95 mg. This represents 500 mg of dextran accounting for the moisture content.

Dissolve 500 mg dextran (allowing for the moisture content) in distilled water and dilute to 500 ml in a volumetric flask. The pre-dried dextran is not used because of the possibility of retrogradation and reduced solubility. This solution contains 1.0 mg of dextran per ml of solution. Dilute 100 ml of this solution to 1000 ml. This solution contains 0.1 mg of dextran per ml. Aliquots of this solution are diluted as follows:

ml stock solution	dilute to	mg/ml
10	100	0.01
20	100	0.02
30	100	0.03
40	100	0.04
50	100	0.05
60	100	0.06
70	100	0.07
80	100	0.08
90	100	0.09
100	-	0.10

Place 2 ml of the 2.5N sodium hydroxide reagent in each of 10 centrifuge tubes. (Centrifuge tubes of the largest size possible compatible with the centrifuge available should be used. Flat bottom, round bottom or cone bottom tubes may be used.) Add 10 ml of a prepared dextran solution (see above) and 2 ml of the copper reagent.

Place the tubes in a boiling water bath for 5 minutes to precipitate the dextran-copper complex. At the end of 5 minutes remove the tubes and allow them to cool 15-20 minutes.

The tubes are then centrifuged at 5000 G for 20-30 minutes to compact the sediment so the supernatant solution can be decanted without disturbing the sediment. If the centrifuge does not go up to 5000G, centrifuge at maximum speed available for a longer time.

The supernatant solution is decanted, 10 ml of the wash solution is added to each tube, and the tubes swirled to suspend the sediment in the solution. The tubes are centrifuged again; the supernatant liquid is decanted and the tubes are inverted on a blotting surface and allowed to drain for 5 minutes. The sediment in each tube is dissolved in 2 ml of 2N sulfuric acid

and the solution is transferred quantitatively to a 10 ml volumetric flask. A second 2 ml portion of 2N sulfuric acid is added to each tube as a wash and this is also transferred quantitatively to the 10 ml volumetric flask. The solution is then diluted to the mark with distilled water.

Phenol-sulfuric acid color development with dextran.

Place 2 ml of each dextran-copper complex solution in a 20 mm x 150 mm test tube. Place 2 ml of water in another test tube for use as a blank. To each tube is added 1 ml of 5% aqueous phenol solution. Then 10 ml of concentrated sulfuric acid is added rapidly all at once from a barrel pipet with a large bore opening so that the acid mixes completely with the aqueous solution producing a maximum of heat. An automatic plastic syringe pipet may also be used to add the acid.

The tubes are placed in a boiling water bath for 2 minutes to insure complete color development^{1/}.

When the solutions have cooled to room temperature (20-30 min) the color is read on a spectrophotometer at 485 nm against the blank prepared with 2 ml of water. The color readings are then plotted on graph paper. If the color is read as percent transmission it must be plotted on semi-log paper of one cycle. If the color is read as optical density or absorbance the values are plotted on square paper, as shown in Figure 1. The curve is used to determine the dextran corresponding to the color measurements in the unknown solutions. The color range should be above 25% transmission, or below 0.6 absorbance units, for accurate readings.

If the color is more concentrated or darker than this range, an aliquot of 5 ml is pipetted from the 10 ml flask of solution that gave too much color and diluted to 25 ml in a volumetric flask. The phenol-sulfuric color test is then carried out with 2 ml of the diluted solution. A dilution factor of five must be included in the calculations.

It is important that the same spectrophotometer cell size be used for both known and unknown solutions.

DETERMINATION

Sugar and Syrups: A sample of 40 g of the sugar (or weight of syrup to contain 40 g sugar) to be tested is dissolved in 60 ml of water and adjusted quantitatively to 100 ml in a volumetric

^{1/} The author is indebted to Dr. Richard Kitchen of B.C. Sugar Refining Company, Vancouver, B.C., Canada for suggesting this modification. This heating step insures completeness of color development.

flask. Then 10 ml of this sugar solution is placed in a 100 ml beaker, and 0.3-0.4 g of analytical filter aid and 40 ml of absolute alcohol are added.

The precipitate which forms is collected with the filter aid on a 15 ml coarse sintered glass (coarse pore size) Buchner filter by vacuum filtration. The precipitate, which contains the dextran, is washed by filling the filter with 80% alcohol five times, allowing each portion to be completely drawn through the precipitate before the next portion is added. Thorough washing is important to remove all sugar from the precipitate.

When the last portion has been drawn through the precipitate, the precipitate plus filter aid is transferred to a 25 ml volumetric flask: this may conveniently be done by first placing a 50 or 60 mm long stem funnel in the empty 25 ml volumetric flask with the end of the stem going to the bottom of the flask. Turn the Buchner funnel containing the filter-aid + polysaccharide precipitate upside down in the long stem funnel, fill the stem of the Buchner funnel with water from a wash bottle, and blow the precipitate and filter aid out into the funnel. The precipitate is broken up with a small spatula and washed into the 25 ml flask. The Buchner funnel is rinsed with water twice, and the washings are allowed to go through the funnel into the volumetric flask. Care is taken not to use too much water and pass the mark on the flask.

The volume is then adjusted to the mark with distilled water and the solution is filtered through a Whatman No. 42, 110 mm fluted filter paper, or by suction on a 15 ml medium, (medium pore size) sintered glass Buchner filter. Another alternative is to filter the solution by suction through a Whatman no. 42 filter paper on a millipore funnel assembly. The dextran is in the filtrate portion of the separation.

The dextran determination is carried out with 10 ml of the filtrate following the procedure described for the standard curve.

Cane Juice: A sample of 50 ml of juice is heated to boiling, and allowed to cool to settle suspended solids. A sample of clear juice is taken and the Brix of the juice is determined. Then 10 ml of juice is placed in a 100 ml beaker, 0.3-0.4 g of analytical filter aid and 1 ml of the 10% trichloroacetic acid solution are added and mixed, and 40 ml of absolute alcohol are added. The same procedure as described for sugar is then followed. The trichloroacetic acid is added only to juice samples to precipitate protein.

Calculation: Calculate dextran, PPM (mg/kg) on solids as follows:

wt. of sample solids diluted to 100 ml = A
 ml of aliquot taken for alcohol precipitation = B
 ml of solution of alcohol precipitate = C
 ml of aliquot taken for copper precipitation = D
 ml of final solution of copper-dextran complex = E
 mg/ml dextran (from standard curve) = F

$$\text{PPM} = F \times E \times \frac{C}{D} \times \frac{1}{B} \times \frac{1}{A} \times 10^5$$

Example: If the dextran read from the standard curve is 0.06 mg/ml and the volumes are as described,

then A = 40
 B = 10
 C = 25
 D = 10
 E = 10
 F = 0.06

Substituting in the formula

$$\text{Dextran} = 0.06 \times 10 \times \frac{25}{10} \times \frac{1}{10} \times \frac{1}{40} \times 10^5 = 375 \text{ PPM}$$

DISCUSSION

It is necessary for the above procedure to be followed precisely and exactly in every detail if accurate results are to be obtained. The copper sulfate stock solution is stable for several months if stored in a dark place but the copper reagent must be prepared each day. The purpose of adding the sodium sulfate to the solution is to speed up the coagulation of the copper complex. The precipitated polysaccharides in the filter aid must be washed by filling the 15 ml Buchner funnel 5 times with 80% alcohol allowing the preceding portion to be drawn completely into the filter mat before adding the next portion. The purpose of this is to remove all of the sugar from the precipitate. The precipitate should not be allowed to sit for an extended time (more than one hour) before it is placed in the 25 ml volumetric flask. If it is allowed to dry out, as it would if left over night, some retrogradation of the dextran may occur which could prevent it from redissolving in water.

The copper-dextran complex is insoluble in alkaline solution but slowly soluble in water. For this reason the sediment in the centrifuge tubes should be washed with alkaline copper sulfate wash solution, rather than water, to remove any traces of sucrose which may be present and would produce added color with the phenol-sulfuric acid reaction.

The phenol-sulfuric acid reaction is extremely sensitive to all carbohydrate materials and great care should be taken to

keep the test tubes and pipet free of dust particles which may contain carbohydrate material. The test tubes used should be exactly the size specified because these large diameter tubes allow the sulfuric acid to mix with the dextran solution rapidly. The color reaction is dependent upon heat. A convenient method of adding the sulfuric acid is to use a pipet bulb with a large bore barrel pipet or an automatic plastic syringe type pipet. The heating step after addition of the acid insures that color development will be complete and results reproducible. The phenol-sulfuric acid does not react with dextran per se, but the hot acid hydrolyzes the dextran to glucose which reacts with the reagent to produce the color. For this reason, this test analyzes for all glucose units in the dextran and therefore the analysis is independent of the molecular weight of dextran used as standard.

Figure 1 shows a standard curve prepared with dextran T40 and T2000 by the copper precipitation method. The molecular weight

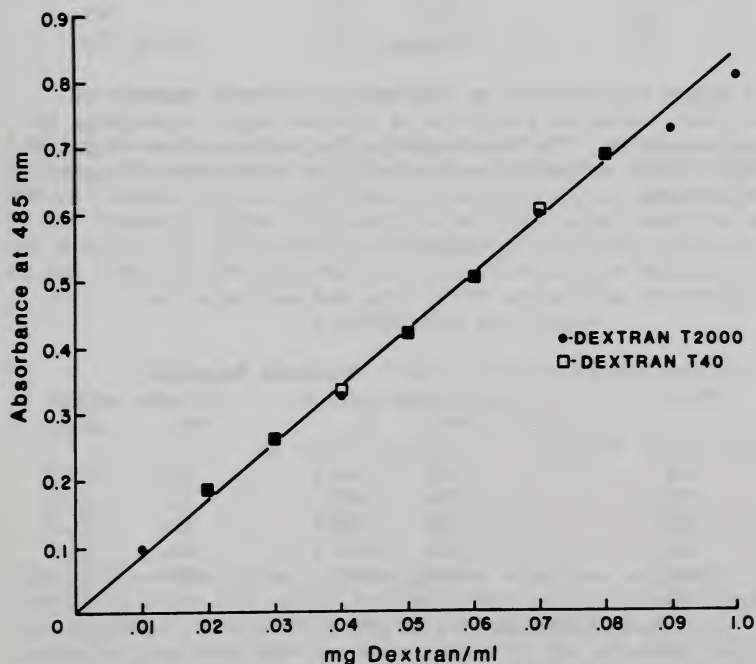


Figure 1.-- Standard curve for dextran.

of the standard does not affect the curve. The curve reads total weight of dextran (mg/ml) vs. absorbance, and therefore there is no difference in the results obtained with the two dextrans of different molecular weight. However, the accuracy of the method does diminish above 0.08 mg per ml; consequently, the samples to be analyzed should be diluted so that the spectrophotometer reading will be between 20% and 80% transmission.

Table 1 shows the recovery of dextrans in known concentrations by the copper precipitation technique. The recovery ranged from 97% to 102% with an average of 99.38%.

TABLE 1

Recovery of Known Dextran T40

Dextran taken mg/100 ml	Dextran found mg/100 ml	Recovery %
0.30	0.29	96.7
0.40	0.41	102.5
0.50	0.50	100.0
0.60	0.59	98.3
Average		99.38

Table 2 shows the recovery by the copper and haze methods of dextran T2000 added to solutions of refined sugar containing no measurable dextran. The recovery by the copper method ranged from 99% to 102% while the recovery by the haze method ranged from 60% to 98%.

TABLE 2

Recovery of Dextran T2000 from Refined Sugar by
Copper and Haze Methods.

Dextran Added PPM	Dextran Recovered			
	by copper method		by haze method	
	PPM	%	PPM	%
375	373	99.5	225	60.3
500	510	102.0	337	67.4
625	620	99.2	524	83.8
750	750	100.0	666	88.8
1000	992	99.2	980	98.0
Average Recovery		99.98%	79.66%	

Table 3 shows the recovery by the copper and haze methods of dextran T40 added to solutions of refined sugar containing no measurable dextran. The recovery by the copper method ranged from 96% to 103% while the recovery by the haze method ranged

from 49% to 73%. The haze values for both the T2000 and T40 dextrans were read from a standard curve prepared with T2000. It is obvious that the recovery of the T40 was slightly lower than that of the T2000 by the haze method. This observation indicates that the haze method gives lower results for dextrans of lower molecular weight than for those of higher molecular weight. The reason is the increased solubility, and decreased precipitation of lower molecular weight materials. Results also indicate that the haze method loses accuracy as dextran levels decrease below 500 ppm.

Table 3

Recovery of Dextran T40 from Refined Sugar by
by Copper and Haze Methods

Dextran Added PPM	Dextran Recovered			
	by copper method		by haze method	
	PPM	%	PPM	%
375	375	100.0	187	49.8
500	516	103.2	300	60.0
625	609	97.4	450	72.0
750	743	99.1	506	67.4
1000	957	95.7	730	73.0
Average Recovery		99.10%	64.44%	

Table 4 shows the effect of soluble starch on the copper method. Two raw sugar solutions were prepared to which 500 PPM of soluble starch was added. The dextran was determined by the standard copper procedure before and after addition of the starch. It is obvious from these results that starch up to the level of 500 PPM does not interfere in the method.

Table 4

Effect of Starch on Copper Dextran Method

Sugar	Dextran PPM	
	no starch added	500 PPM starch added
1	1844	1846
2	388	387

Table 5 shows the effect of indigenous sugarcane polysaccharide (ISP) on the copper method. Two raw sugar solutions were prepared to which 500 PPM of ISP was added. Dextran determinations were made on each sample by the standard copper procedure before and after addition of ISP. It is obvious that ISP up to 500 PPM does not interfere in the determination.

Table 5

Effect of Indigenous Sugarcane Polysaccharide (ISP)
on Copper Dextran Method

Sugar	Dextran PPM	
	no ISP	500 PPM ISP
1	1093	1091
2	337	330

Table 6 shows a comparison of the dextran values obtained when typical raw sugar samples are analyzed by the copper method and the haze method. The results show that the results obtained by the copper method are considerably higher than those by the haze method. This may be due in part to the effect of molecular weight of the dextran in the raw sugars and the variation in completeness of precipitation in 50% alcohol with molecular weight. Because the copper method determines total dextran present, it was expected that results by this method would be higher than these from haze analysis.

Table 6

Comparison of Copper Method and Haze Method for Dextran

Sugar	Dextran - PPM	
	CuSO ₄	Haze
1	337	114
2	270	0
3	1000	443
4	388	262
5	1844	1598
6	1065	578

Table 7 shows a comparison of the copper and haze methods in another series of raw sugars. The dextran determinations were made in duplicate by the copper method. The haze method values on the same samples were determined by the refinery that supplied the raw sugar samples (a sponsoring company of S.P.R.I.). Here again the values obtained by the copper method are considerably higher than those of the haze method except in the case of sugar number 1 which is probably erroneous.

Table 7

Comparison of Copper Method and Haze Method for Dextran

Sugar	Dextran PPM	
	CuSO ₄	Haze
1	375	726
	375	
2	750	309
	780	
3	750	581
	750	
4	462	328
	475	
5	1000	882
	1000	
6	859	619
	843	
7	294	300
	300	
8	578	337
	588	

Table 8 shows the results of seven repeat determinations made on aliquots of the same sample of sugar. The mean value was 1004 PPM, the standard deviation was ± 19.6 , and the coefficient of variation was 1.95%.

Table 8

Repeatability of Copper Method on a Raw Sugar Sample

Replication	Dextran found PPM
1	1000
2	1031
3	1000
4	1031
5	984
6	1000
7	984

Mean = 1004

Standard deviation = ± 19.6

Coefficient of variation = 1.95%

It is hoped that this test will be simplified with further development. It must again, however, be emphasized that the current procedure must be followed exactly for accurate and reproducible results.

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DISCUSSION

H. R. PRIESTER (Savannah): Do you know a mathematical relationship between ppm dextran and the error in polarization that the dextran will cause.

E. J. ROBERTS: No, I do not.

M. FOWLER (Amstar): In the copper precipitation part of the test, is this precipitation quantitative in regards to copper?

E. J. ROBERTS: No, not necessarily. There may be other things in there that will precipitate the copper, but will not form a color in the final step of the determination. You cannot base a dextran determination on the copper in the precipitate.

M. FOWLER: Have you run recoveries on spiked samples of raw sugar in lieu of refined sugar?

E. J. ROBERTS: Yes, we have. Those results will be given tomorrow at the SIT meeting.

M. A. CLARKE: In regard to Mr. Priester's question, this mathematical relation between ppm dextran and pol error can be calculated from the specific rotation of dextran which is +199 as compared to +66.53 for sucrose.

T. BALOGH (Redpath): Did you run this test on other than raw and refined sugars; for instance, syrups or molasses?

E. J. ROBERTS: Yes, we did. Those details are also in the next paper.

G. VANE (Tate and Lyle): You mentioned diluting if you want to measure at 485 nm. The absorption spectrum of the phenol-sulfuric color has a nice peak at 485, but going toward the blue, it has a nice wide peak of about one fifth the OD at 485 nm just at 420 nm. So, one could consider measuring at 420 if one had a sugar colorimeter. Then you would not need a spectrophotometer at all.

E. J. ROBERTS: We will have to look into that. Thank you.

C. C. CHOU: Have you conducted a collaborative study on this test method?

E. J. ROBERTS: We are doing that right now. We invite everybody to join this collaborative study.

A. HO (Redpath): The method calls for adding about 0.3 to 0.4 g of filter aid, and add everything to a 25 ml volumetric flask. Suppose that I use 0.3 and somebody else uses 0.4, will this not change the concentration of the final solution?

E. J. ROBERTS: The change will be so little that it will be insignificant.

A. HO: Do you experience any problem with higher amounts of dextran, such as 1000 ppm, exhausting the phenol sulfuric acid reaction.

E. J. ROBERTS: You could surely accommodate a sample of over 20,000 ppm dextran without any problem of exhausting the phenol sulfuric acid.

A. HO: Does the presence of heavy metals in the raw sugar effect the analysis?

E. J. ROBERTS: I have not added any heavy metals, but I do not anticipate any problem because there is plenty of excess copper if the heavy metals precipitated. Heavy metals do not form any color in the phenol sulfuric acid reaction.

C. GOODACRE (Tate and Lyle): In tables 6 and 7 you refer to results by the haze method and you also have some recoveries

studied with the haze method. There is no description of what particular variant of the haze method is used, or what calibration curve was used.

E. J. ROBERTS: The haze method that was used is the one described in the Cane Sugar Handbook (Meade-Chen). It is the ICUMSA method. The same method was used for all results reported here.

J. A. POLACK: Have you tried the method on molasses, syrups or juices?

E. J. ROBERTS: Yes, we have, and those results are in the next paper.

DEXTRANS, DEXTRAN HYDROLYSIS PRODUCTS AND VISCOSITY IN SUGAR SOLUTIONS

Frederick W. Parrish

U.S. Department of Agriculture

INTRODUCTION

High molecular weight dextrans have long been known as products of deterioration of sugar cane and of sugar juice brought about by bacteria of the genus *Leuconostoc*. The effects of these polysaccharides on sugar processing have been reviewed (Imrie and Tilbury, 1972), and include effects on clarification, evaporation and crystallization rate of sucrose, factory capacity, and exhaustibility of massecuites. A common factor in these effects is the increase in viscosity of the sugar solution due to the presence of dextrans. The effect of dextrans on the viscosity of sugar solutions and molasses has been examined in a shear viscometer (Greenfield and Geronimos, 1978) and in a capillary viscometer (Geronimos and Greenfield, 1978). In this study the viscosities of sugar solutions and the effects of dextrans have been determined with an oscillation viscometer (Oppliger, Matusik, and Fitzgerald, 1975), which has some advantages for sugar liquors.

Another effect of dextrans is on crystal shape of sucrose resulting in elongation along the c-axis (Keniry, Lee, and Davis, 1967). The dextran content of sugar liquors can be reduced by treatment with dextranase enzyme (Tilbury, 1971), and such treatment under processing conditions has been shown to improve the filterability of sugar juice (Fulcher and Inkerman, 1974). The products of the action of dextranase enzyme on dextrans invariably include isomaltose, and it has been suggested that isomaltose, rather than dextran, is responsible for the crystal habit modification observed as elongation along the c-axis (Kelly and Mak, 1977). This postulate has been examined as part of this study.

EXPERIMENTAL

Materials

Dextrans of 500,000 and 2 million molecular weight, determined by light scattering, were obtained from Sigma Chemical Co. Native dextran was prepared in our laboratory. These three dextrans were products of the growth of Leuconostoc mesenteroides NRRL B-512 on sucrose. Total carbohydrate analysis on dextrans was performed by the phenol-sulfuric acid method standardized using anhydrous D-glucose (Dubois et al., 1956). Analytical reagent grade sucrose (Fisher Scientific Co.) used in this study was shown by the alkaline copper method (Roberts, 1982) to contain less than 10 ppm dextran.

Methods

Viscosities of sucrose solutions at intervals of 5° Brix from 45-65° Brix and at intervals of 5°C from 35-75°C were determined with an oscillation viscometer, Model 7.006 (Nametre Co., Edison, NJ). The instrument was zeroed in air, and calibrated with water. Similarly, viscosities of sucrose solutions were measured in the presence of 6500 ppm (solution basis) of the three dextrans and isomaltose. Densities were measured using calibrated 10-ml pycnometers.

Sonication of 65° Brix sucrose solutions (100 g) containing 0 or 6500 ppm dextran (mw 2 million) was performed for 5-15 minutes at 60°C at 200 watts and 20KHz using a Braunsionic Model 1510 sonicator (B. Braun Melsungen AG).

Reducing sugars were measured by the dinitrosalicylic acid method (Miller, 1959).

Native dextran (20 g) in 0.1M sodium acetate buffer pH 5.8 (1000 ml) was incubated 16 hours at 40°C with dextranase (Intl. Enzyme Co., Troy, VA) from a Penicillium species. The digest was deproteinized by the addition of 5% zinc sulfate (100 ml) followed by an equivalent amount of 0.2M barium hydroxide solution (Somogyi, 1945). The resulting precipitate was removed by centrifugation, and the sugars in the protein-free supernatant solution were fractionated by charcoal chromatography (Barth and Timell, 1958). High performance liquid chromatography (hplc) of a sample of the enzyme digest was performed at 34°C on a column of Aminex HPX-87 (Biorad) with 7mM sulfuric acid as eluent.

Sucrose was crystallized at 60°C for 24 hours from a solution (100 g), saturated with sucrose at 80°C, in the presence of 0 or 6500 ppm native dextran or isomaltose. Seed crystals (10 mg) were used, and the round bottomed flask was rotated continuously on a Buchi Rotavapor (Brinkmann) at about 30 rpm. Crystals were separated by centrifugation, washed rapidly with

95% ethanol, and dried at 65°C in vacuum. The crystals were measured on a Zeiss binocular polarizing microscope containing a graticule in the eyepiece, and photomicrographs were made.

RESULTS AND DISCUSSION

Detailed investigations on the effects of dextrans on the viscosity of sucrose solutions have been reported (Greenfield and Geronimos, 1978; Geronimos and Greenfield, 1978) which enable the calculation of viscosity of sucrose solutions containing dextran of various molecular size and for various concentrations of dextran and sucrose. The instruments used in the above investigations were a rotating cylinder viscometer and a capillary viscometer. Such instruments have some limitations for use in the industrial environment of sugar processing. For example, capillary viscometers are laborious to clean when used with sugar solutions, and particulate matter in the solutions interferes with measurements. These disadvantages do not pertain to an oscillation (or vibrating sphere) viscometer, used in our laboratory over the past 6 years, in which the power required to maintain a constant-amplitude oscillation is transformed to a digital display of viscosity x density. Measurements can be made with high precision and high sensitivity on 35-ml samples in a portable unit (Natl. Bureau of Standards, 1972) over the range 10^{-1} to 10^5 centipoise. Measurements are made at the same shear rate, can be obtained at the rate of 1 sample per minute provided temperature equilibrium is attained, and give identical readings to those obtained by capillary viscometers. The instrument is also available in an on-line version. A possible disadvantage of the oscillation viscometer is that the readout is a product of viscosity (cps) and density (g/ml) necessitating pycnometer measurements of density if viscosity readings are required. However, for comparison purposes in practical situations the "product" readout may suffice since viscosity changes are of greater magnitude than corresponding density changes (Table 1).

Table 1.--Comparison of Nametre readout with viscosity for sucrose solutions at 65°C.

Sucrose (wt%)	Density (g/ml)	Viscosity (cps)	Density x Viscosity
49	1.199	3.10	3.72
60	1.260	8.17	10.3
70	1.321	31.0	41.0

The close correspondence between viscosity measurements made with the oscillation viscometer and the capillary viscometer is shown for sucrose solutions at 35°C (Table 2). Having

Table 2.--Comparison of viscosity determinations on sucrose solutions at 35°C using different instruments

Sucrose (wt%)	Viscosity (cps)	
	Capillary	Oscillation
45	5.41	5.38
50	8.36	8.35
55	14.1	14.0
60	26.5	26.5
65	58.0	58.1

established the accuracy of the oscillation instrument for pure sucrose solution, measurements were extended to sucrose solutions containing 6500 ppm dextran (solution basis). The dextran concentration was based on solution concentration rather than ppm on Brix because it is the absolute concentration of dextran which is of importance from a rheological standpoint (Greenfield and Geronimos, 1978). The dextrans derived from Leuconostoc mesenteroides NRRL B-512 were chosen for study because of the readily available dextrans, this type has fewest anomalous linkages, i.e., other than α 1,6 linkages, and its structure appears well established (Figure 1) (Larm, Lindberg, and Svensson, 1971). Viscosity measurements on sucrose solutions (45-65° Brix) containing 6500 ppm B-512 dextran (mw 2 million) from 35-75°C are shown (Table 3, Figure 2). Corresponding data with dextran (mw 500,000) are given also (Figure 2), and it can be seen that a semilog plot gives an essentially linear relationship between viscosity and temperature (Figure 3). This relationship has been established previously (cited in Greenfield and Geronimos, 1978).

Preliminary experiments were performed on the sonication of 65° Brix sucrose solutions containing 0 or 6500 ppm dextran (mw 2 million). After treatment for 15 minutes at 60°C the viscosity of the sucrose solution containing dextran decreased approximately 5%. An effect of similar magnitude has been observed on sonication of starch (Azhar and Hamdy, 1979). No formation of reducing sugars was detected under these conditions, although it has been shown that ultrasonic radiation can convert sucrose into monosaccharides (Szent-Gyorgyi, 1933). These preliminary results do not indicate a practical value of ultrasonic treatment in reducing viscosity due to dextrans in sugar liquors, but the possibility remains that

ultrasonic treatment could be applied to inactivation of enzymes in cane sugar juice.

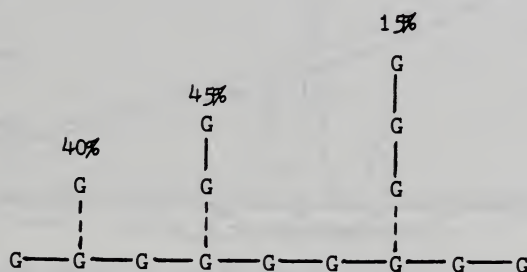


Figure 1-- Structure of B-512 dextran

— α 1,6 linkages
 --- α 1,3 linkages

Table 3.--Viscosity-temperature data for sucrose solutions containing 6500 ppm B-512 dextran (mw 2 million).

Temp (°C)	Sucrose (wt%)				
	45	50	55	60	65
35	12.8	18.8	25.3	48.8	110
40	11.2	15.7	21.7	41.4	94.0
45	9.5	13.2	18.5	35.1	80.0
50	8.0	11.0	15.9	29.7	68.1
55	6.7	9.2	13.6	25.2	57.9
60	5.7	7.7	11.6	21.4	49.3
65	4.8	6.4	9.9	18.1	42.0
70	4.1	5.4	8.5	15.4	35.7
75	3.4	4.5	7.3	13.0	30.4

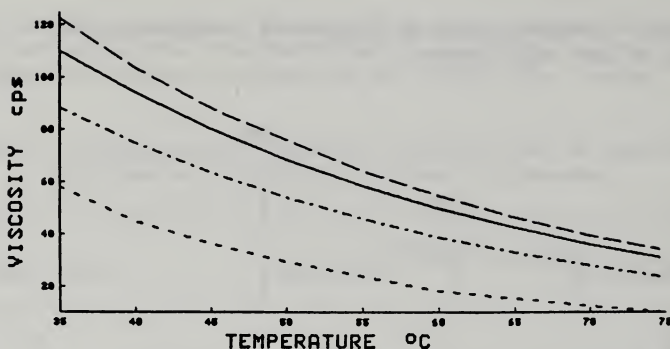


Figure 2--Viscosity of sucrose (65 Brix) + dextrans

- Sucrose
- Sucrose + 6500 ppm dextran mw 500,000
- Sucrose + 6500 ppm dextran mw 2 million
- Sucrose + 6500 ppm native dextran

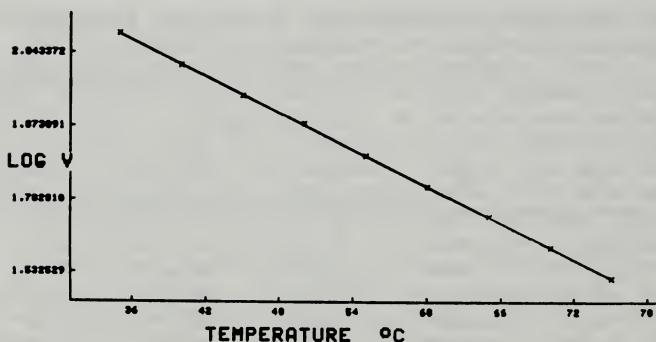


Figure 3.—Semilog plot of viscosity of sucrose (65 Brix) + 6500 ppm native dextran vs. temperature.

It has been established that the action of dextranases from *Penicillia* on B-512 dextran gives \underline{D} -glucose, isomaltose, and isomaltotriose (Tsuchiya, et al., 1952). When B-512 native dextran was treated with a dextranase from a *Penicillium*, the products were shown by hplc to consist of the above three sugars together with a fourth sugar, judged from its retention time to be a trisaccharide (Figure 4). Since the anomalous linkages in B-512 dextran are α 1,3-linkages (Larm et al., 1971), the second trisaccharide from the enzymic hydrolysis of the dextran is probably 3²- α -glucosyl isomaltose. The relative amounts of the products are shown (Table 4).

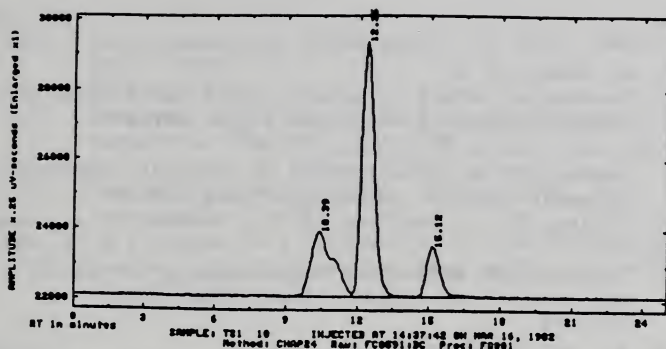


Figure 4.—HPLC of separation of products from hydrolysis of native dextran with dextranase.

Table 4.--Relative amounts of the products from dextranase enzyme on B-512 dextran

Product	Retention time (min.)	Amount (%)
D-Glucose	15.12	13
Isomaltose	12.35	60
3 ² - α -Glucosyl isomaltose	10.98	9
Isomaltotriose	10.39	18

Isomaltose was isolated from the enzymic hydrolysis of B-512 native dextran by charcoal chromatography using an aqueous ethanol gradient (Barth and Timell, 1958). When 6500 ppm of isomaltose was added to sucrose solutions the effect on viscosity was not different from that of an equal amount of additional sucrose. Sucrose which was crystallized at 60°C from solutions containing 6500 ppm isomaltose showed none of the c-axis elongation which was observed with sucrose crystals produced from solution containing 6500 ppm native dextran.

In summary, this study has established that oscillation viscometry can be applied to sugar liquors, and that isomaltose does not bring about c-axis elongation of sucrose crystals.

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DISCUSSION

K. ONNA (H.S.P.A.): A number of our factories process cane infected with up to 10% smut (a cane disease). There is also an increase in the dextran level. The juice purity appears higher than it ought to be. There is a problem in sugar boiling. And, there is an accumulation of elongated grain particularly in the low grade system. You have mentioned the image analyzer. Could you elaborate a little more on what your plans might be? This is one that our people would be interested in.

F. W. PARRISH: The image analyzer is an instrument manufactured by Cambridge Instruments. The model we have costs \$150,000; the latest model costs \$80,000. It was purchased for some very serious studies we have going on a lung disease called byssinosis produced by cotton dust. Also grain dust explosions are devastating, even by military standards. We are very much involved in trying to combat these grain dust problems. This is why we have this rather expensive instrument. It is a microscope connected to an interactive dedicated computer system that will measure crystal diameters, areas, perimeters, length to diameter ratios, or almost whatever you want to do. The computer analyzes whatever image you can find with the microscope. I would like to look at the crystal size, and the elongation ratios. I have given some thought to looking at the dynamics of crystallization with the image analyzer. You make a motion picture film and go to the image analyser frame by frame. This should be a very simple matter to do, and it has been done in other industries.

ANON: Can you identify the crystallography with the image analyzer.

F. W. PARRISH: No, you cannot. But in addition to the image analyzer, we have optical microscopy and polarizing microscopy.

A. B. RAVNO: I am interested in whether you can extend the range of the Nametre viscometer to study high consistency, non-Newtonian fluids. When we get down to low purity in a raw

sugar mill, we are dealing with essentially non-Newtonian fluids. The kind of viscosities that are involved are vastly higher than the 50 to 100 centipoises that you were considering. We are considering 500,000 centipoises or higher.

F. W. PARRISH: Yes, certainly much higher viscosities can be measured, although it is outside the range of the instrument that I have. It is, however, a constant shear viscometer which limits its application to non-Newtonian fluids.

E. J. CULP: In low purity materials, the viscosity is a function of time. A freshly made solution will increase in viscosity over several hours due to the regrouping of molecules forming hydrated clusters. Have you encountered anything like that in your measurements with dextran?

F. W. PARRISH: No. We get a response that becomes constant within one minute, and remains constant indefinitely. By indefinitely we mean the 15 minutes that we have observed the viscometer readout. For thixotropic materials the viscosity will change and these changes are observable with this viscometer.

G. VANE: I had not budgeted for an image analyser and I wanted to use something like a light pen. For only about \$1500 you can buy a "graphics tablet". This can be hooked up to a suitable computer and with this you can just run the light pen over your photomicrographs. The results are about the same as with an image analyser for 1/100 the price.

F. W. PARRISH: Yes, that works. I have done it that way, too. It is laborious. That is a disadvantage.

FOAM FORMATION IN SUGAR LIQUORS - ITS ORIGINS AND RELATIONSHIP TO SURFACE TENSION

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Tate & Lyle Ltd.

INTRODUCTION

Traditionally, foaming in sugar liquors during processing has been predominantly associated with beet sugars. Polypeptides and saponins are documented (Dutton and Oldfield, 1967) as the major causative agents in beet sugar systems. Foam prevention has been said to be the largest single process aid cost in beet sugar processing (Tjebbes, 1980).

In contrast, foam formation has been of little or no consequence in cane sugar processing and thus has received little attention in the past. An exception to this is, perhaps, foaming of cane molasses in storage (referred to often as "froth fermentation") which can cause problems to cane raw sugar producers. Recent studies in Barbados (Brooks and Smith 1980) suggest that amino acid levels may provide a means of predicting the frothing potential of molasses.

Recently, we were provided with a sample of cane raw sugar which had caused an instance of foaming in a cane sugar refinery. This sugar had been on melt for a period of weeks and the foam observed in process was certainly associated with this raw sugar. Foam was observed at the mechanical strainers but not in the recovery (remelt) process. Loss of decolorisation performance at the bone char station was also observed whilst this sugar was on melt. No process additives of a surface active nature were in use, nor had recently been, in the refinery at the time of this observation. Since this incidence of foaming, we have received another sample of a cane raw sugar that had shown significant foam in process - more than usual, but less than that described in the foregoing.

Given these samples of cane raw sugars of different foamabilities, and the ready availability of non-foamers, we decided to investigate the relationship of foaming characteristics to surface tension in these cane

raw sugars. We have included some examples of beet raw sugars as comparisons.

PREVIOUS WORK ON FOAMING IN CANE SUGAR SYSTEMS

Only in recent years has any work been published in this area. Hiroshi et al (1980) carried out measurements of foamability on cane raw sugars and some study of the causative agents. They concluded that a steroid saponin was mainly responsible for foaming. They found no correlation between foaming, and the nitrogen content of fractions responsible for foaming, whereas Cheng et al (1981) found a correlation between foamability and protein content, for a large number of foaming cane sugars. They also reported that traces of amylose ($> 100\text{ppm}$) would enhance the foaming, above a certain level (0.1%) of protein.

The latter accords with the results of Dutton and Oldfield who concluded that high molecular weight peptides in beet sugar systems would induce foaming, and questioned the role of saponins in foam formation. They were unable to identify a low molecular weight foaming component also present in the systems studied.

THE PRESENT WORK

The observations of foaming and reduced decolorisation performance were, at first sight, consistent with the presence of surface active impurities. The absence of foam in the recovery process could be due to selective occlusion of some or all of the surface active impurities in the sucrose crystals, but since they would also be present in the molasses film, it is far more likely that the low purity of the molasses breaks the foam. This is qualitatively consistent with our observation that higher purity raw sugars show enhanced foamability, which was also reported by Hiroshi et al.

EXPERIMENTAL WORK

After examining documented chemical tests for detecting surfactants, some tests were selected for cationic, anionic and non ionic surfactants (see Appendix 1 for details) and applied to the cane raw sugar which had shown high foamability in the refinery.

Surface tension measurements were carried out on a selection of cane raw sugars, beet raw sugars and triple washed granulated sugar. These measurements were made using a Willhelmy plate tensiometer, which is described in Appendix 2. Three cane raw sugars were selected as examples of high foamability (A), of medium foamability (B) (on the basis of the refinery observations previously described) and of low

foamability (C), a West Indian raw that showed no tendency to foam in the refinery process), and other cane and beet raws were also examined. Surface tension was measured at intervals, up to two hours, for each sample, on 40° Brix solutions in distilled water.

Foamability was evaluated by gassing sugar solutions under defined conditions of gas purity, flow rate, solution concentration and temperature. For full details see Appendix 3. The sugar solution under test was gassed under standard conditions and the foam height measured at the point when the gas was shut off. Its collapse was followed at suitable time intervals.

The raw sugars A, B, C and D (see on) were analysed for total nitrogen content and for the more common amino acids. The latter were determined only as a total; that is, the free and combined amino acids.

RESULTS

The chemical tests used all gave negative results.

Surface tension results are shown in Figure 1. For comparison, solutions of granulated sugar and of triple-washed granulated sugar, were also measured. All of the raw sugars, and the granulated sugar, showed reduction of surface tension compared to the purest sample used. The beet raw sugars form a group below the cane raw sugars, but the cane raw sugars of varying foamability do not fall in any obvious order. The lower initial surface tension values in the case of the beet sugars, and the similarity of all of the raw sugars in their surface tension/time profiles, suggests that the surface active species present in the beet sugars is the more active, and that the molecular size of the surface active species is similar in all cases. The rate of attainment of the lowest surface tension observed, for a particular solution, depends upon the rate of diffusion of the surface active species to the surface and the formation of the surface structure.

Whilst the pH values of these sugar solutions were not widely different, the surface tension measurements were repeated on some of the sugars at a range of pH's; firstly, to eliminate any effect of pH on surface tension, and secondly to see whether different sugars displayed different changes with changes of pH. Sugars A, B and C, and a beet raw sugar (D) were examined at pH's of 5, 7 and 9 as 40° Brix solutions. The results are shown in Figures 2 - 7; the surface tension observed initially are shown in Figure 2, those after two hours in Figure 3, and Figures 4 - 7 show the time dependence of surface tension for each sugar at the different pH's studied.

FIGURE 2
10 SEC. SURFACE TENSION

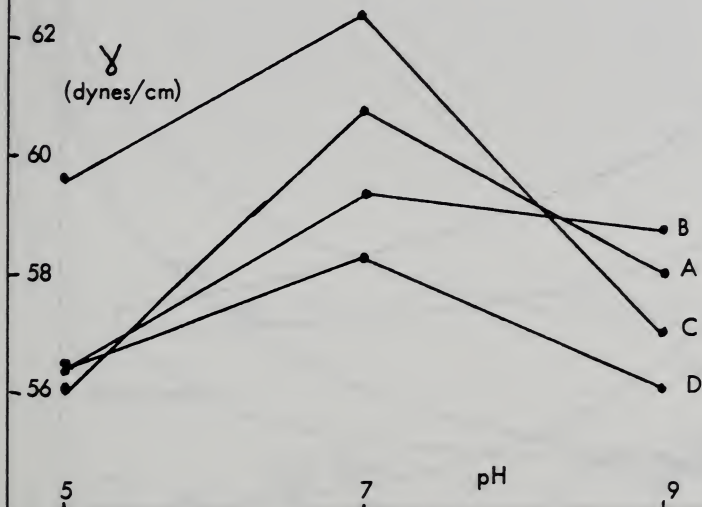


FIGURE 3
2 HOUR SURFACE TENSION

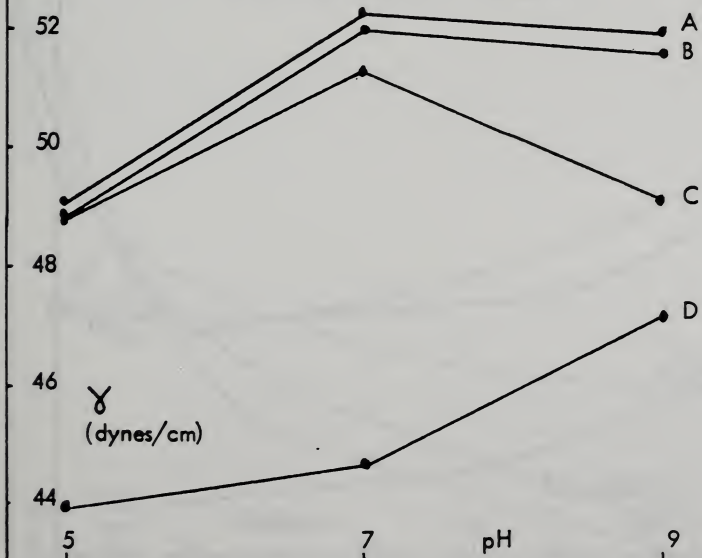


FIGURE 4
SURFACE TENSION - SUGAR A

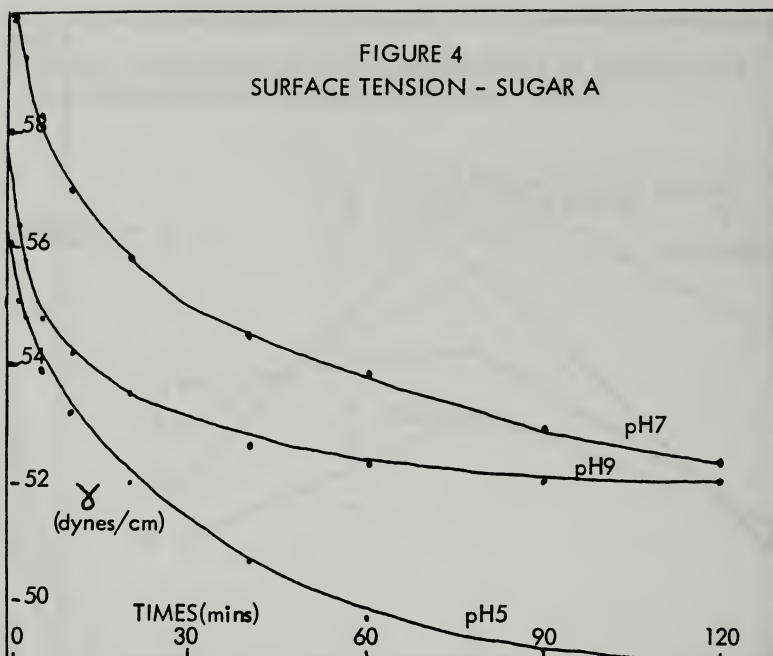


FIGURE 5
SURFACE TENSION - SUGAR B

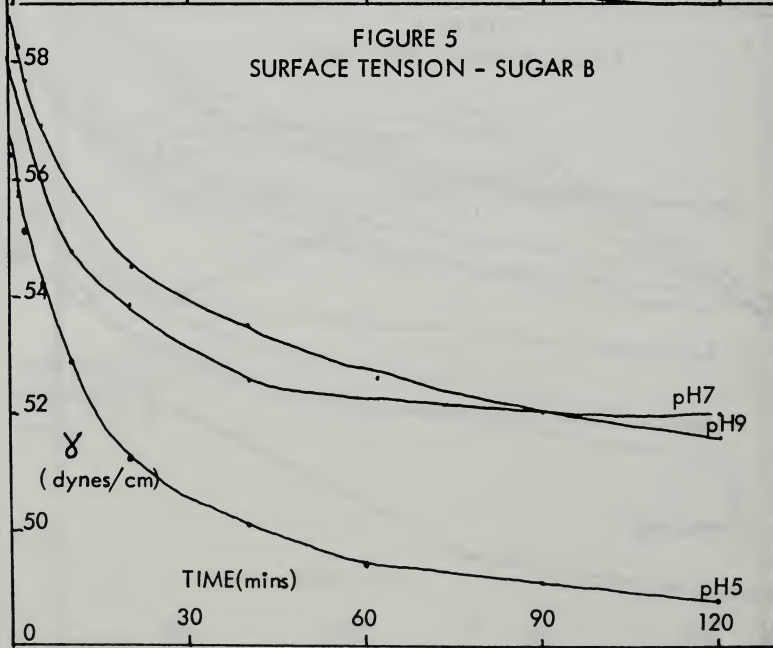


FIGURE 6
SURFACE TENSION - SUGAR C

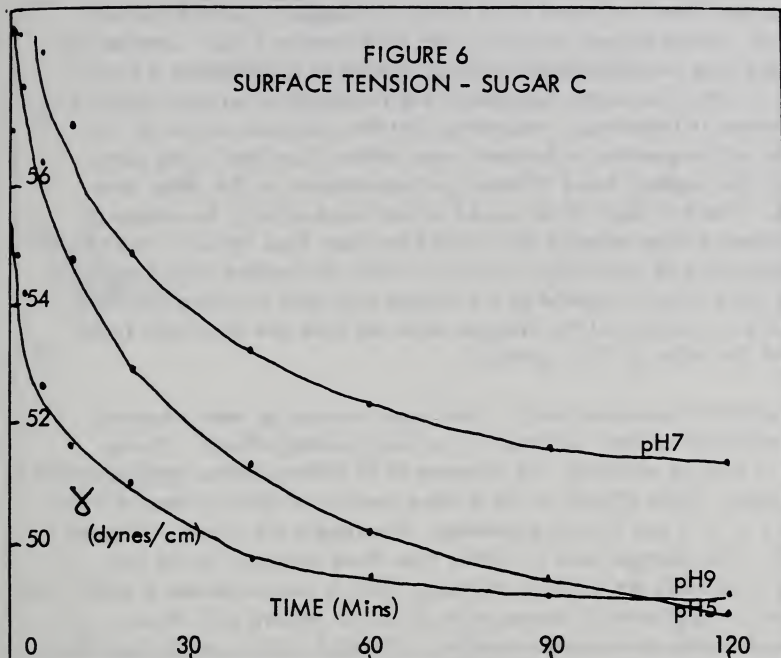
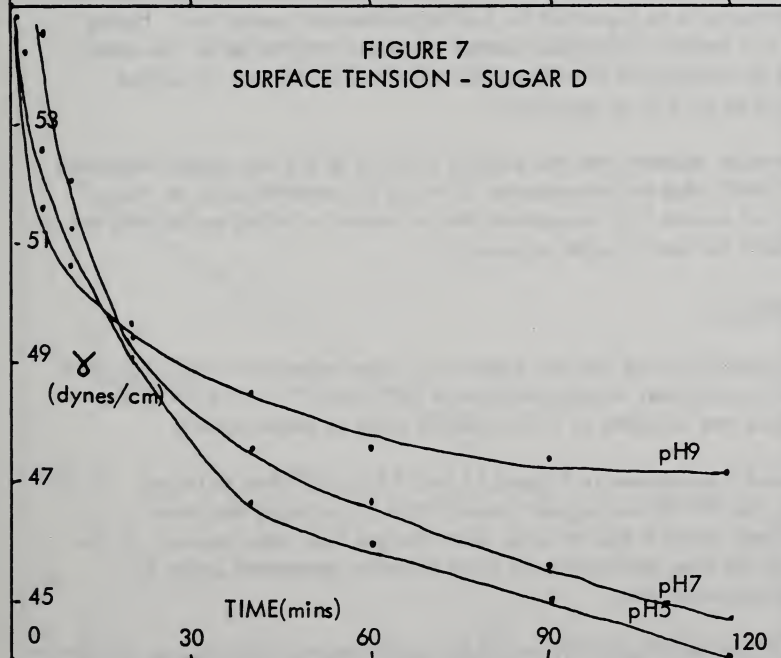


FIGURE 7
SURFACE TENSION - SUGAR D



It is evident that the sugars show differing changes in surface tension with pH, which themselves differ with equilibration time. Considering the zero time values, the variation of surface tension follows a similar pattern in the four sugars. However the two hour values show definite differences in behaviour, indicating that the chemical nature of the surface active species in the beet sugar differs from that in the cane sugars; also sugar C has a different pH dependence to the other cane sugars. The two hour values would reflect more closely the nature of the surface active material than would the zero time values - apart from the magnitude of the surface activity - since the surface active agent(s) would have time to migrate to the surface and form a surface structure. It should be noted that the changes observed here are relatively small, only of the order of a few dynes.

Two surfactant products used in cane sugar processing were selected, and examined for their surface tension and foaming effects. Hodag CB-6 (a boiling additive) and Busperse 49 (a surface active antiscalant) were used. Their effects on the surface tension of 40% sucrose solutions at pH's of 5, 7 and 9 were examined. The results are shown in Figures 8 - 10. The changes with pH differ from those observed in the raw sugars examined; the activity of Hodag CB-6 is much reduced at pH9. Further, the patterns of change with pH do not accord with those observed for the raw sugars examined. The high activity of Hodag CB-6 in particular is as expected for such a commercial surfactant. Hodag CB-6 is a methyl glucoside coconut oil ester, and the ester side chain would be removed at alkaline pH's. Therefore, the loss of surface activity at pH 9 is as expected.

It therefore appears that the surface activity of the raw sugars examined is not solely due to the presence of traces of materials such as these, though of course it is recognized that a number of other surfactants are available for use in sugar processing.

Foamability

The foamabilities of the raw sugars A-D were examined in 60° Brix and 30° Brix solutions, at temperatures of 25° and 50°C. The pH of the solutions was adjusted to 7.0 (at 25°C) prior to measurement.

The results are shown in Figures 11 and 12 for 60° Brix solutions. In all cases, the 30° Brix solutions formed little or no foam; the latter collapsed within a few seconds when the gas flow was stopped. In the case of the beet raw sugar, no foam could be generated under the conditions used here.

The 25° values (Figure 11) are higher than those at 50° (Figure 12) but

FIGURE 8
2 HOUR SURFACE TENSION VALUES
HODAG CB - 6 (10 ppm)

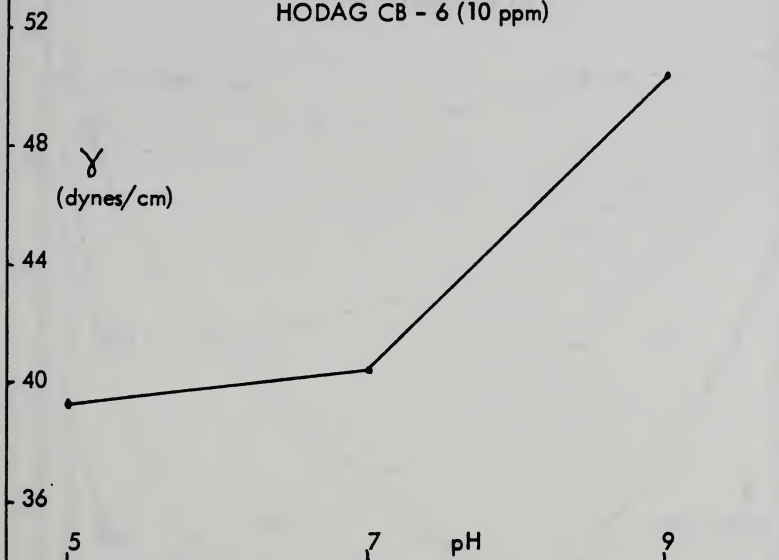


FIGURE 9
2 HOUR SURFACE TENSION VALUES
BUSPERSE 49 (50 ppm)

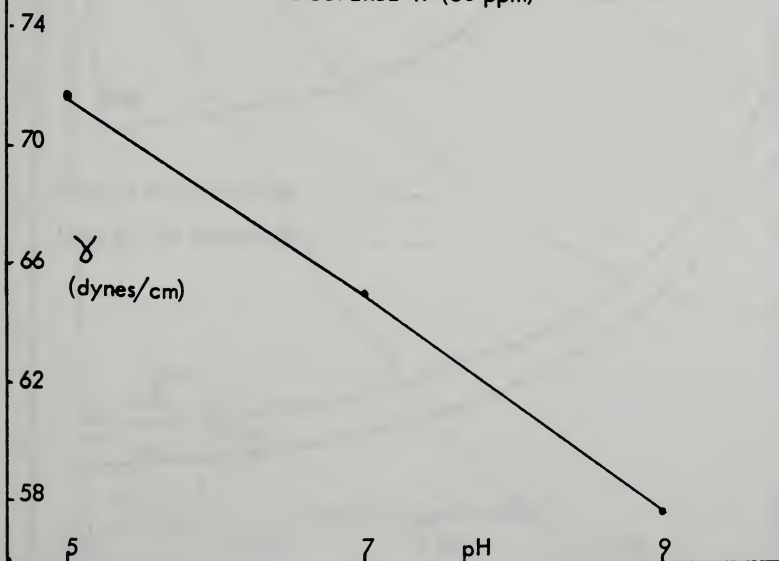


FIGURE 10
SURFACE TENSION OF SURFACTANTS
AT DIFFERENT pH's

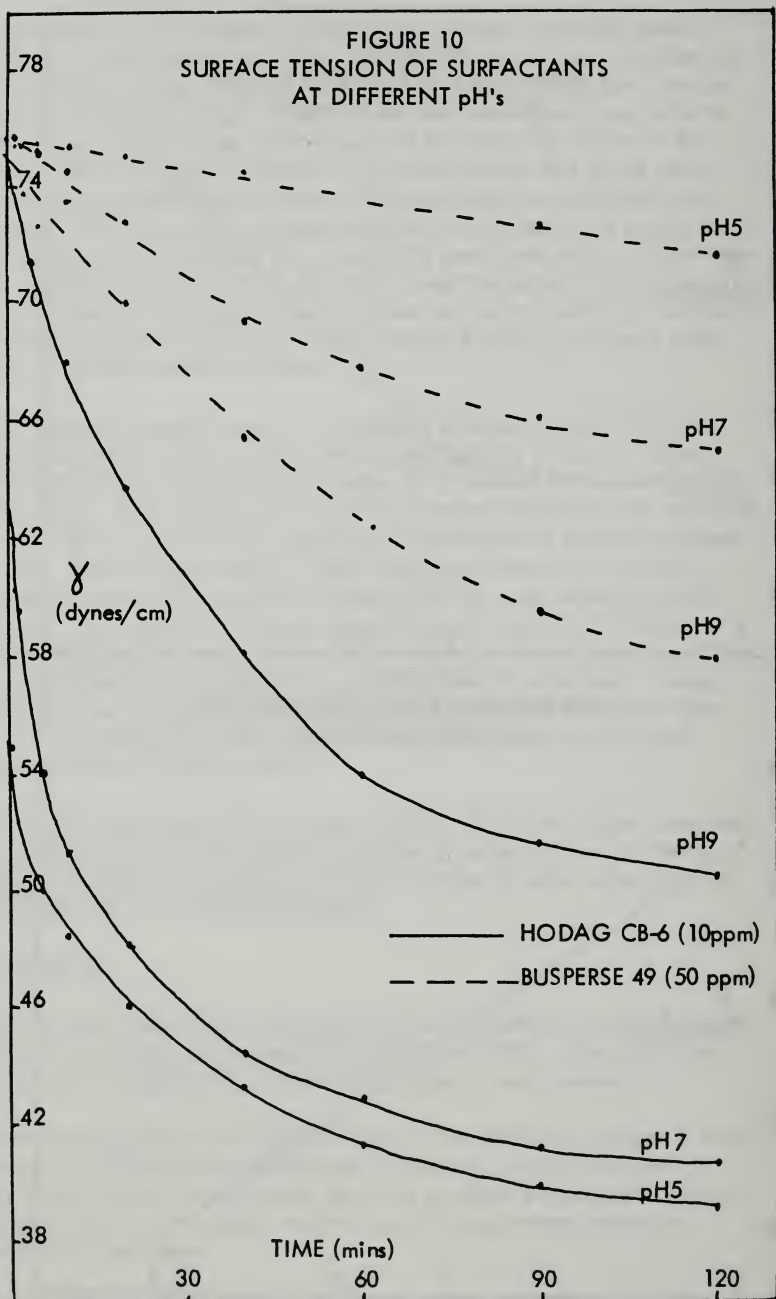


FIGURE 11
FOAMABILITY AT 25°, 60° BRX

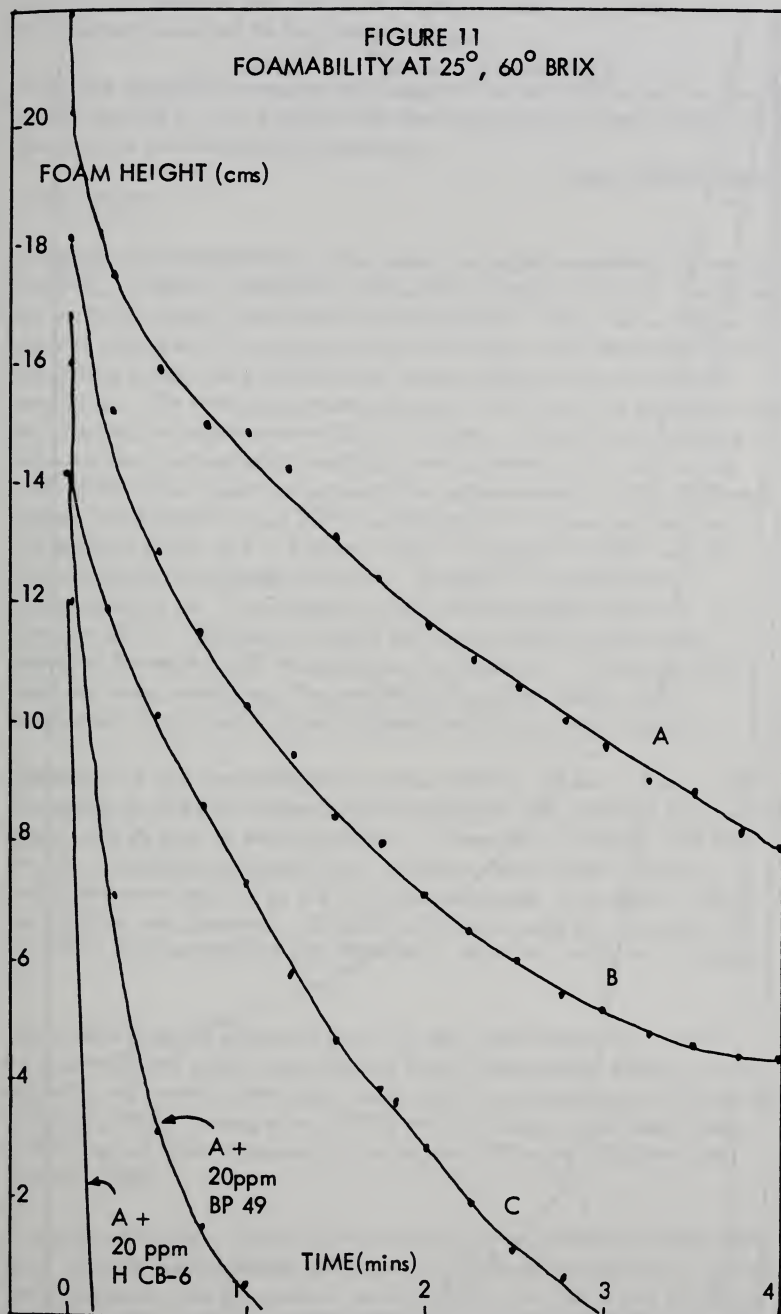
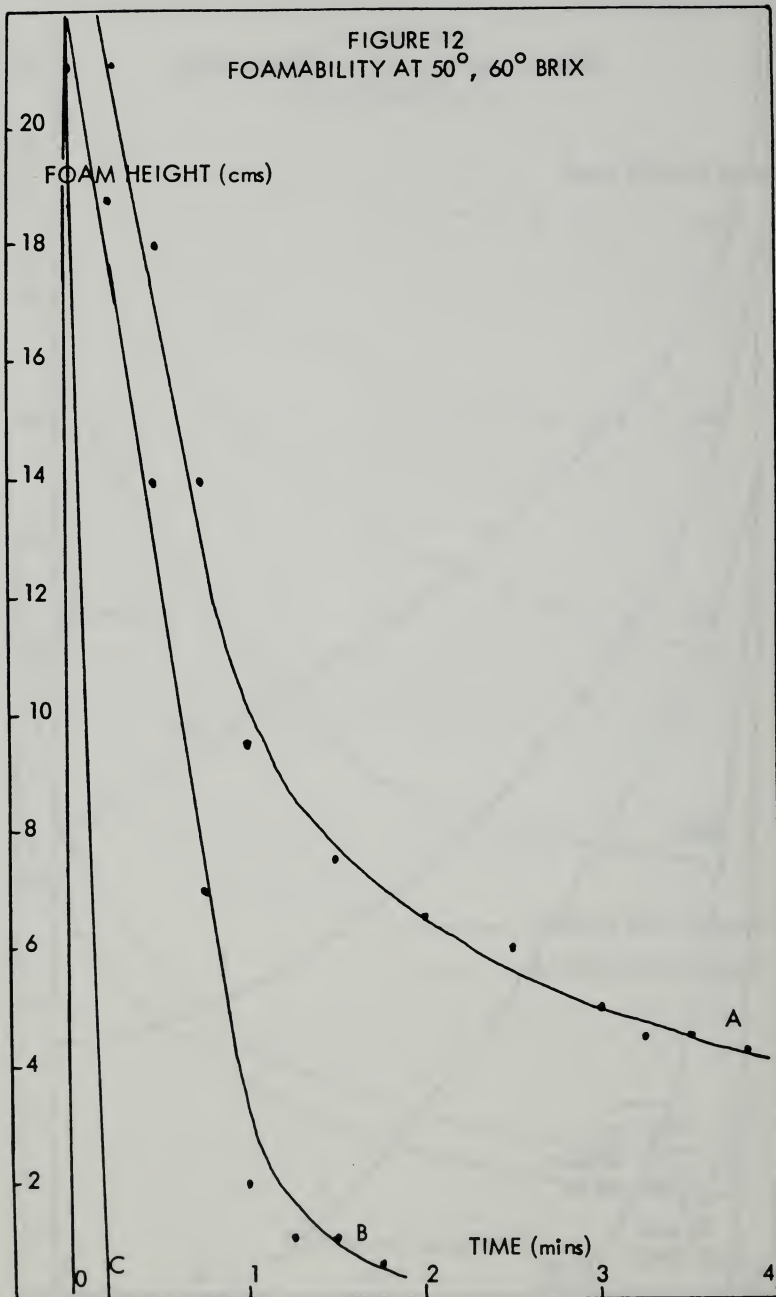


FIGURE 12
FOAMABILITY AT 50°, 60° BRIX



both lie in the order that would be expected from the refinery observations reported in the introduction.

When the foamability results are compared to the surface tension results at 25° and pH 7, it is evident that the equilibrium surface tension values decrease as the foamability decreases.

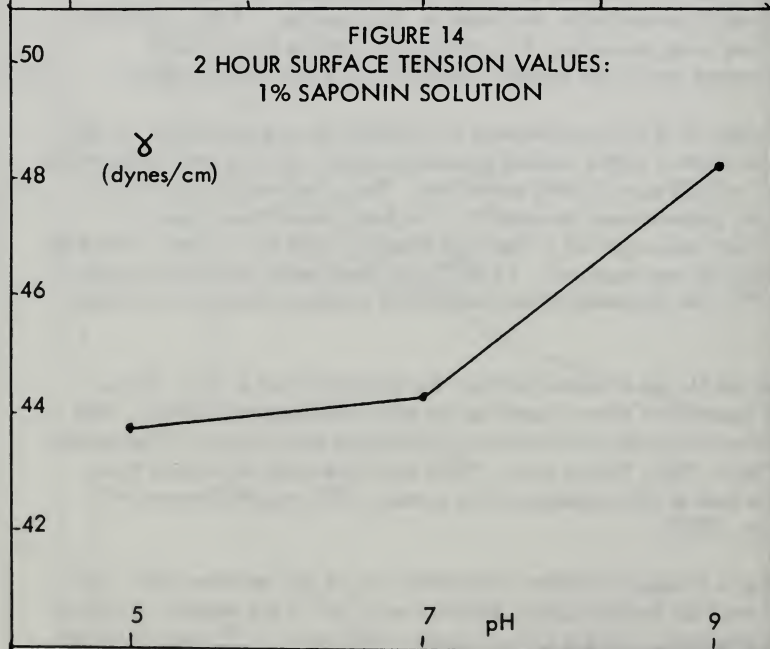
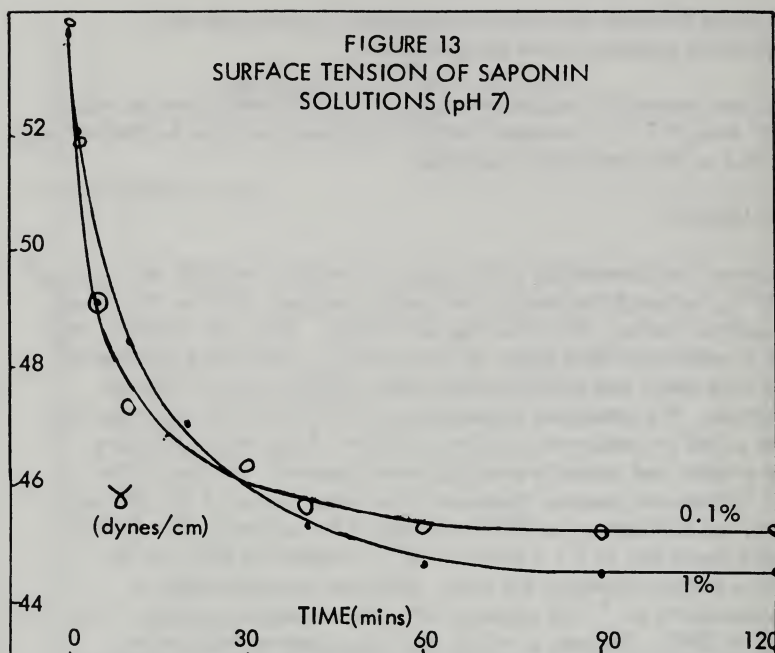
DISCUSSION

It appears that foamability in the cane raw sugars examined, as measured at 50°C, is directly related to the surface tension. That is, the lower the surface tension, the lower the foamability. Thus, for example, raw sugar C exhibited little foam, of low stability, under the experimental conditions used, and exhibited the lowest surface tension under all conditions. We attempted measurements at 50°C but the apparatus used is not suited to measurements above ambient. Evaporation problems were evident and unsteady readings were observed. In view of the small differences observed between the surface tension of the different sugars, we decided to use 25°C as standard for surface tension work. We did find a fall of 2 - 3 dynes at 50°, compared to 25°, but the relative pattern remained the same. Whereas we could attain a reproducibility of ± 0.2 dynes at 25°, this worsened to nearly ± 1 dynes at 50°C. The rate at which the lowest surface tension was achieved increased with temperature, as expected. If the practical problems were overcome, the variation of surface tension with temperature may yield further information in these investigations.

The effect of the two surfactants on foamability was examined. A 60° Brix solution of triple washed granulated sugar was prepared and portions dosed with 20 ppm of each surfactant. These test solutions, and the control, showed zero foamability. Further, when these doses of surfactant were applied to the high foaming sugar A, a drastic effect on foamability was observed. At 50°C, no foam could be induced at all. At 25°C, the foamability was drastically reduced, as shown in Figure 11.

These results are as expected from the data obtained so far. So we have to examine other impurities for foam enhancement effects. Both saponins and protein have been implicated in previous work (Dutton and Oldfield, 1967, Cheng et al, 1981) and have been mentioned along with a host of other compounds in a recent SPRI report (Roberts and Clarke, 1982)

A sample of saponin (Sigma Chemicals) and of α -amylase (BAN 120L) were used for further study. Aqueous solutions of the saponin in buffers were prepared, and the surface tension profiles at 0.1% and 1% (w/w) solutions were measured. The results are shown in Figures 13 and 14,



and it is immediately evident that the surface activity of this sample of saponin is only a fraction of that of commercial surfactants such as CB-6. The variation of surface tension with pH shows a marked similarity to that of beet sugar D (see Figure 3). This is qualitatively in accord with expectations for beet sugars, but suggests that saponins are not the prime cause of surface activity in the cane raw sugars studied. This contrasts with some previously published work on foaming in cane sugar systems (Hirashi et al, 1980).

Nitrogen analysis of the raw sugars showed significant differences; the beet raw sugar, as expected, is higher in nitrogen than the cane sugars. We also included two other cane raw sugars which had shown no foamability in process, and also showed virtually no foamability under the experimental conditions used here.

The results are shown in Table 1:

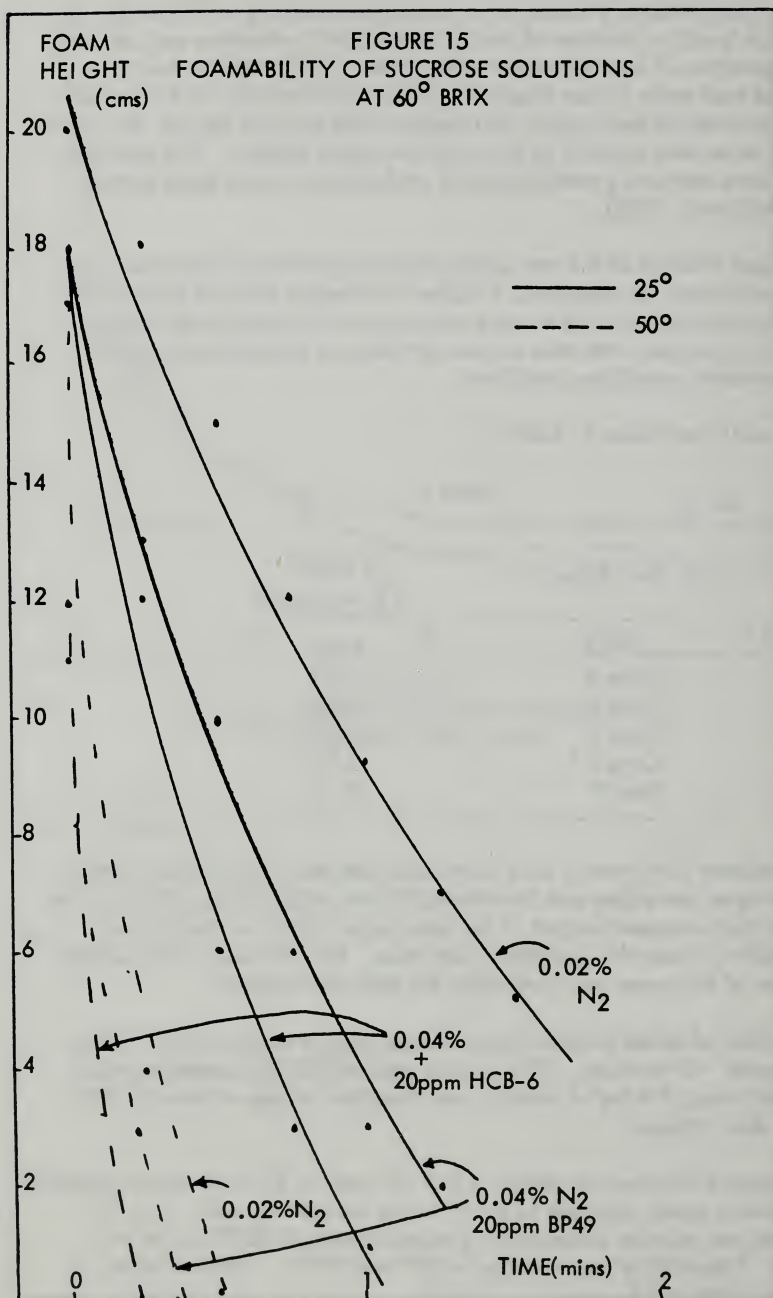
Table 1.

Raw Sugar	Nitrogen (% on sample)
Cane A	0.06
Cane B	0.03
Cane C	0.02
Cane E *	0.02
Cane F *	0.01
Beet D	0.11

It is evident that there is some correlation between the nitrogen content of the cane raw sugars and the foamability as measured here; but in spite of the high nitrogen content of the beet sugar it did not display any foamability under the conditions used here. But, of course, the surface tension of this sugar was lower than the cane raw samples.

The effect of added protein was examined using a sample of BAN 120L, a common α -amylase. This enzyme preparation was quoted as being approximately 3% (w/w) protein; and therefore represents about 0.5% of soluble nitrogen.

The enzyme solution was added in 0.2 ml doses to 25 ml of triple-washed granulated sugar, adjusted to pH 7, in the foamability cell. 1 ml of the enzyme solution corresponds to approximately 0.025% (w/w) on sugar. Foamability was measured at 25° and 50°C. The results are shown in Figure 15.



As expected, the sugar solution showed zero foamability. However, as the enzyme concentration was increased, foam could be induced by nitrogen gassing. It became significant at a nitrogen level of 0.01% and at 0.02% gave foam that showed some degree of stability, especially at the lower temperature. Foamabilities were similar at 25° and 50°, but at the higher temperature the foams were less stable. This was as observed for the cane raw sugars studied in this work.

The dependence of foamability on pH was examined using sugar A, as a 60° Brix solution, at 25°. Portions of the solution were adjusted to pH's of 5, 7 and 9, and the foamability of each was measured. The foam height, and its collapse were indistinguishable at the lower pH's, but the height and stability were considerably enhanced at pH 9. The foam formed in the latter case was visibly different, the bubble size being smaller; and a lesser tendency for the bubbles to coalesce with resultant foam collapse. In view of the observed change in surface tension with pH (Figure 3) this observation further suggests the importance of other factors besides surface tension in foam formation. At 0.04% addition of nitrogen, enhanced foamability was observed, with enhanced stability. As expected, the addition of 20 ppm of the surfactants reduced the foamability significantly, as shown in Figure 15.

The effect of 0.025% and 0.05% doses of the enzyme on the surface tension of 40° Brix solutions of triple-washed granulated sugar was examined, at a pH of 7. From a value of 75 dynes, the observed surface tension fell to 70.5 after ten minutes, with 0.05% nitrogen. With 0.025% nitrogen, the initial value of 76 fell to 72 after ten minutes. These results indicate very low surface activity for this enzyme in sugar solutions; and the values observed at zero time indicated some significant increase in surface tension. This has been previously reported for aqueous amino acid solutions (Wyman et al 1936).

The amino acid profiles were determined for the raw sugars A - D. The most abundant amino acids in all cases were aspartic and glutamic acids, and alanine and glycine. A similar preponderance of these amino acids was observed in the α -amylase used, which also showed a similar level of histidine. The levels of these amino acids found are shown in Table 2.

These amino acids are all common to a variety of natural proteins and the levels show no particular correlation to foamability. This is perhaps not unexpected since their actual incorporation in proteinaceous material may well differ between the different sugars.

From the results obtained so far, it would appear that proteinaceous material can be a primary cause of foaming in cane raw sugar liquors. This is in accord with comparative experiences in the cane and beet industries, even though the beet raw sugar examined in this work did

Table 2.

Amino Acid	Sugar A	Sugar B (in μ moles/100 mg sample)	Sugar C	Sugar D	BAN 120L (μ moles/ ml)
Aspartic acid	1.26	0.51	0.57	0.28	40.6
Glutamic acid	0.28	0.17	0.11	2.26	33.0
Alanine	0.23	0.13	0.18	0.13	17.8
Glycine	0.12	0.09	0.16	0.13	20.0
Histidine	0.01	0.01	0.01	0.01	24.0

not exhibit significant foamability. The observed effects of the surfactants added on foamability shows that other factors besides surface tension influence foamability. It is suspected that surface viscosity is an important factor which we have not examined in this work. Sugar concentration plays an important part in foamability, as measured in this work, as does temperature.

The effects of these two parameters on foamability are in accord with viscosity being an important factor.

ISOLATION OF SURFACE ACTIVE MATERIALS FROM RAW SUGARS

For details of the procedures used, see Appendix 4.

Surface tension measurements before and after treatment of solutions of the sugars with powdered carbon, showed that the surface active materials were completely removed from solution. We then attempted to isolate the surface active materials from the carbon by extraction with hot 95% ethanol. Anticipating that significant amounts of sucrose would be obtained along with the surface active and other impurities, we next separated the sucrose using a nonionic resin treatment on the evaporated ethanol extract. Following this treatment of sugars A - D, a small quantity (0.05 - 0.2%) of the original weight of sugar) of yellow solid was obtained from each sugar.

These solids from sugar B were subjected to proton NMR and IR spectroscopy. The spectra all looked very much like those of sucrose. The NMR showed some faint peaks around 1.5 ppm, where aliphatic protons would appear. The IR spectrum showed no discernible peaks besides those of sucrose, with the blurring of peaks characteristic of contaminated samples. We concluded, therefore, that the extracted material

was mainly sucrose. However, in all cases, the extracts were markedly surface active.

The resin separation was repeated, which reduced the weight of isolated material by ca 50% in each case. In view of the small amounts of material being obtained after the repeated separation steps, further studies were carried out using thin layer chromatography.

Silica TLC plates were used, with an aqueous alcohol mixture as eluant, and the plates developed with carbazole reagent (see Appendix 4 for details). On standing, this reagent develops a dark coloration with carbohydrates. Sucrose and saponin were run, with samples of the four extracts prepared from sugars A - D. The chemical surfactant tests (see Appendix 1) were used on each of the extracts. Using TLC, we were unable to detect anything besides sucrose in extracts A, C and D; B showed some material of R_f greater than sucrose; the saponin sample used proved to be a mixture, seven spots being developed; no corresponding spots were evident in sugar D. Further, the chemical tests all gave negative results.

From these results we concluded that the impurity (or impurities) responsible for surface activity in the sugars studied are present in very low concentrations, and therefore must have a significant specific surface activity. A more specific means of extraction, and/or of separating sucrose from the impurity extract is required. A large quantity of sugar sample is required, to afford more extracted material for study.

CONCLUSIONS

Whilst surface tension plays a part in determining foamability in cane raw sugar liquors, foam may be suppressed by the addition of surface active agents. The formation and stability of foam, for a given raw sugar, is strongly dependent upon concentration and temperature; it is highly probable that surface viscosity is an important parameter. A sample of protein studied proved to be a strong foam promoter, whilst not being very strongly surface active. The surface active impurities in the raw sugars studied appeared to be present in very small amounts, and are therefore of high specific surface activity. No evidence of saponins being involved in cane systems was found, but the surface tension results were consistent with saponin being a prime cause of the surface activity of the raw beet sugar studied.

APPENDIX 1

Chemical Tests for Surface Active Agents

(i) General Colourimetric Tests (Goebel Test)

This test utilizes colour and fluorescence changes with a mixture of dyes.

Reagents: Methylene blue (10g/100ml water)
 Uranine (0.5g/100 ml water)

Procedure: 0.1 ml of each reagent added to 10 ml of 50° Brix solution of sugar; or to 1 ml of 10% solution of extracted surface active material in water

Anionic
Surfactant: green fluorescence generated

Cationic
Surfactant: vivid blue/red colour generated

Nonionic
Surfactant: dark blue colour, little fluorescence

(ii) Specific Tests

(a) Anionic Surfactant

Reagent: 5 ml of 0.005N hydrochloric acid containing three drops of 0.1% thymol blue.

Procedure: 1 ml reagent added to 1 ml of 60° Brix sugar solution, or 0.1 ml of 10% solution of extracted material in water.

Anionic Surfactant gives a red-purple colouration

(b) Cationic Surfactant

Reagent: 7.5 ml 0.2N sodium acetate solution, 10 ml acetic acid and 2 ml 0.1% Bromophenol blue (dissolved in 96% ethanol), pH adjust to 3.8

Procedure: 60° Brix sugar solution, or 10% aqueous solution of extracted surface active material, adjusted to pH 7; several drops added to 10 ml of the above reagent.

Cationic Surfactant gives a sky-blue colour.

(c) Nonionic Surfactant:

Reagent: 17.4 g ammonium thiocyanate and 0.2 g cobalt nitrate dissolved in 100 ml water

Procedure: 10 ml of 60° Brix sugar solution, or 1 ml of 10% solution of surface active material; add 10 ml of reagent, stand for two hours at ambient temperature.

Polyoxyethylene Surfactants form blue solution.

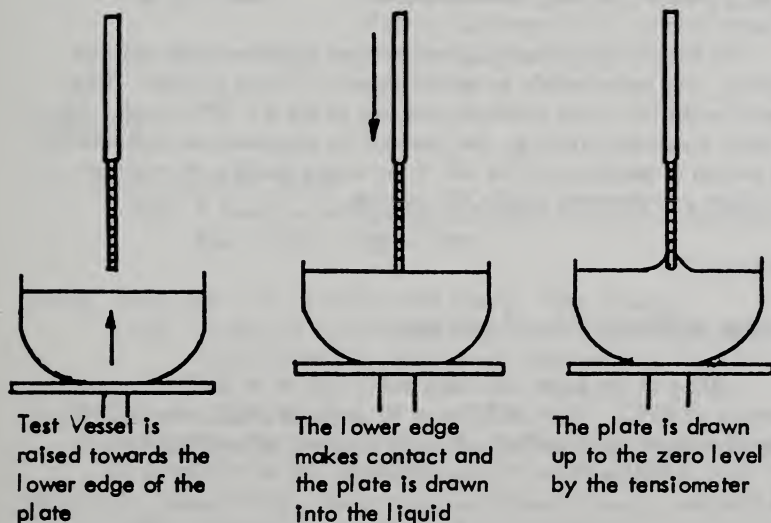
APPENDIX 2

Surface Tension Measurements

A Krüss Tensiometer was used for surface tension measurements at the air/liquid interface. This was used with a platinum plate, and the sample container thermostatted 25° C ($\pm 0.1^\circ$ C). A torsion balance measures the force required to balance the downward pull due to interfacial surface tension on the lower edge of the plate.

Prior to use, the plate is thoroughly cleaned with acetone, and finally cleaned in a flame.

Separate stages in measurements with the plate (the plate is shown in section) are:



Because the lower edge of the plate is brought to the plane of the liquid surface, there is no additional hydrostatic effect requiring correction. Differences in wetting angle are very small and are ignored in calculation of surface tension.

Surface tension (γ), is calculated from the formula

$$\gamma = k \text{ (tensiometer reading)}$$

where k is a constant at a given temperature.

APPENDIX 3

Foamability Measurements

Measurements were made at 60° and 30° Brix sugar solutions, at pH values indicated, using a jacket glass column of length 250 mm and 20 mm internal diameter. Water was circulated through the jacket from a thermostatted ($\pm 0.1^\circ\text{C}$) bath. 25 ml of sugar solution was used for each foamability run; the solution was gassed via a sintered frit in the base of the column, using pure nitrogen. A scale graduated in millimeters was attached to the side of the column.

The sugar solution was gassed for 45 seconds, at a flow rate of 1200 ml/min.; the gas was shut off, and the foam height recorded. The collapse of the foam was followed at suitable time intervals, and recorded as height above the original liquid level.

The foam height, for a particular sugar solution under defined conditions, was reproducible to approximately ± 5 mm of foam. Thus, the reproducibility of the measurements was in the 5 - 10% range. For the freshly prepared solutions, the foamability observed was independent of the period of gassing, but for all of the sugars studied, a maximum foam height was observed within 45 seconds.

APPENDIX 4

Extraction of Surface Active Impurities

600 g of the sugar was dissolved in 400 ml of distilled water, by heating at 60°C. After addition of 30 g of activated carbon (BDH powdered carbon, acid washed), the solution was left stirring for one hour. 10 g of Celite filter aid was added and the solid filtered off under vacuum.

These solids were washed with cold water to remove entrained sucrose solution, and the air dried solid material were stirred for one hour with 500 ml of 95% ethanol. After filtering off the solids, the extraction was repeated. The combined ethanol extracts were evaporated under vacuum, at 40° C, to yield a yellow syrup.

Impurity Enrichment

A nonionic resin was used to separate sucrose from impurities in the extract. Duolite ES861 (25 ml) was placed in a column, and washed with 250 ml of methanol, 250 ml of acetone and finally with 250 ml of water.

The extracted material was dissolved in 100 ml of water at 60°C, and added to the resin in a suitable flask. This was stirred at 60°C for one hour. The resin was filtered off and stirred with 100 ml of isopropyl alcohol at ambient temperature. This extraction was repeated and the combined alcohol extracts evaporated off under vacuum. The solids were finally left overnight under vacuum.

Thin Layer Chromatography

Running solvent:	5 volumes	1-butanol
	3 volumes	absolute ethanol
	2 volumes	water
Spray reagent:	0.1 g carbazole in 95 ml absolute ethanol. 5 ml concentrated sulphuric acid added after carbazole was dissolved.	

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J. Amer. Chem. Soc., 58 1851 - 1856

DISCUSSION

T. BALOGH (Redpath): We did have a problem with foaming. If we got a sugar from one country, we got one type of a problem. If we got a sugar from a different country, we got a different type of a problem. So, foaming depends on the origin of the sugar. We don't know what the mill is doing with the cane, juice, or sugar or what is carried over. But, it certainly gives us a problem. It starts at the melter. It foams again in the carb tanks when the gas is bubbled through the liquor. The pumps start cavitating, we are unable to form a chalk; the pH stays up; the sweetland presses die, and we have to reduce the melt. We did send a sample to SPRI. But, we don't really know whether or not the mills are holding something back on us; maybe they could help us.

J. A. POLACK: I have only seen foaming once in a raw juice, and this was from very badly soured cane. It was very high in dextran content. It was part of our dextranase studies. The juice was foaming badly in a tank in which we were heating it. The addition of dextranase immediately dropped the foam. This suggests that in that case dextrans were responsible for the foam. In your paper, I did not understand how surface tension changed with time. What is the time axis? What is time zero?

G. W. VANE: Time zero is as fast as it could be added and measurements could be started. It is probably 30 or 40 seconds. The reason the surface tension changes is because the solution is homogeneous when you start the measurements, but with time the surface active species migrate to the surface. In some cases they coat the plate which gives funny results, but you don't get that unless you have a really high dose of something surface active. We did not have that problem until we began to measure commercial surfactants. I think that the effect of dextran is to increase the viscosity which causes more foam. The effect of temperature and concentration on viscosity is in agreement with this. I did some experiments adding indigenous cane polysaccharide. This charged polysaccharide has a straighter structure and would probably be a better viscosifier than dextran. However, I could not find any change in foamability. But, what I didn't do was look at surface tension.

M. A. CLARKE: With regard to the beet sugar that you could not get to foam, was that at any particular pH? Did you try to form foam at a high pH?

G. W. VANE: No, I only did that at pH 7, I simply ran out of time to extend the measurements to other pH's on this sugar.

M. A. CLARKE: In regard to the saponins; you found no effect of saponins on foam formation, although the Japanese and others have reported in the literature that saponins do cause

foam. We know that there is saponin like material in cane, including the apigenins mentioned by Dr. Peter Smith in his paper at this conference. Why do you think that you found no effect?

G. W. VANE: There are different kinds of saponin, and we used what happened to be around. It came from a root crop but not sugar beets. The Japanese were basing their work on a rather blurred infrared spectrum. IR's of impure compounds tend to be blurred. Their IR looked very much like ours from our impure extract. We used NMR and other things and could only find sucrose. There was only traces of something else that could have been saponin, at 1.5 ppm in the proton NMR. TLC showed no indication of saponin.

M. A. CLARKE: When we examined certain sugars that gave foaming problems, proton NMR, showed traces of long chain fatty acids. This type of compound is added both as viscosity-reducing agents in the raw sugar factory and as detergent or bactericide. This may have been a case where too much compound was added or where some vessel was incompletely rinsed.

T. BALOGH: So, it does come from the mill.

A. B. RAVNO: You asked for comments on the importance of foam. T. Balogh has certainly given some in the refinery context. I take it that your studies were centered around the high brix levels encountered in a refinery?

G. W. VANE: We looked at 30, 40 and 60 brix.

A. B. RAVNO: Well let me offer a few comments on the problem of foaming in the lower brix ranges. Severe foaming can occur on occasions with mixed juice in the 12 to 15 brix range. This could be due to the presence of some surface active material in the juice. In South Africa we have for some time been concerned that certain problems in juice clarification could possibly also be related to the presence of certain surface active agents which possess the property of stabilizing some of the colloidal and finely dispersed materials, thereby preventing the formation of a good settling floc. We have found that it is possible to induce refractory behavior in a juice sample by the addition of surface active materials. But when we tried to correlate poor clarification behavior with surface tension, as you did for a foaming tendency, we got no meaningful tie up.

One final comment - final molasses is often sold to the fermentation industry where it is diluted to lower brix levels before use. They also encounter severe foaming problems at times in their fermenters due to some component in the molasses.

BIOCIDE VERSUS DEXTRAN IN SUGARCANE MILLING

James C. P. Chen, Consultant, B. A. Smith, U.S. Department of Agriculture, and J. J. Molina, W. R. Crowley Sugar House

INTRODUCTION

It is known that the effective application of biocide in sugarcane milling can reduce the formation of dextran (Chen 1964, Meade 1977). It is recognized that by reducing the production of dextran, which is produced from sucrose, there will be a reduced loss of sucrose in juices from the mill, before the mixed juice is pumped to the boiling house. The question is, how to correlate the use of biocide to the quantification of dextran formation and concomitant sucrose loss.

In order to assess the dextran formation, a method of quantitative determination has to be established. Sucrose loss can be analyzed gravimetrically.

The current procedure for dextran analysis is the haze assay, (Meade 1977), but the amount of haze changes with different molecular weights of dextran (Keniry 1969, Richards 1976, Chou 1980), and also changes with different concentrations of alcohol, time of development, and other variables (Chou 1980).

Because of the importance of dextran, particularly of the low molecular weight range around 40,000, which affects the operational efficiency in a sugar refinery, as reported by Chou and Wnukowski (1980), this study will examine dextran of molecular weights 40,000 and 70,000 in biocide treatment tests, and will observe the correlation of the pattern of sucrose loss to the molecular weight distribution of dextran in the treated and untreated juices.

EXPERIMENTAL

Establishment of Quantitative Method for Dextran

Because a simple spectrophotometer, such as Hach model DR-EL/2, is generally available in a raw sugar factory for

boiler-feed water analysis, the first step was to use this type of instrument to establish a standard curve for each molecular weight of dextran, using the highest available wavelength of 700 nm and a cell width of 2.54 cm as specifications available on this sort of instrument. The CSR Haze test (Meade, 1977) uses a wavelength of 720 nm, but this is above the range of most simple spectrophotometers.

(A) Chen's Modified Method.

In order to establish a method that a raw sugar factory with limited laboratory resources can easily adopt for routine analysis, Chen adopted the ICUMSA method (Haze test), including the processes of removing starch with amylase, ash with cationic and anionic ion exchange resins, and protein with trichloroacetic acid (ICUMSA, 1976, Meade, 1977). A haze development time of 20 minutes was chosen before reading for absorbance on a spectrophotometer at 700 nm. Figure 1 shows results from two research laboratories, USDA in Weslaco, TX and Olin in Kansas City, KS. Reproducibility appears satisfactory at the new wavelength of 700 nm.

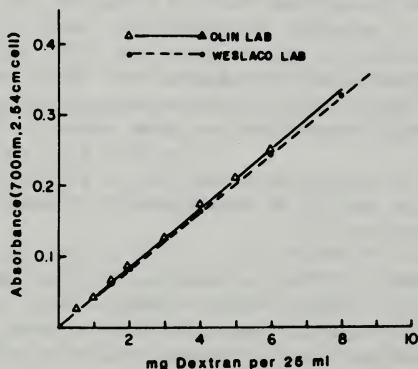


Figure 1.--Dextran calibration graph (mw 70,000).
(Add absolute alcohol to mark, stand 20 min.)

A change in method of addition of alcohol was instituted. Alcohol was added, 1:1, to the test solution, instead of being added to fill a 25.0 ml flask to the mark. Figure 2 shows standard curves of dextran 40,000 mw and 70,000 mw using this method. The line of 70,000 in figure 2 was on a lower level than the same in figure 1, confirming the findings obtained by Chou and Wnukowski (1980) about increasing haze with increasing concentration of alcohol.

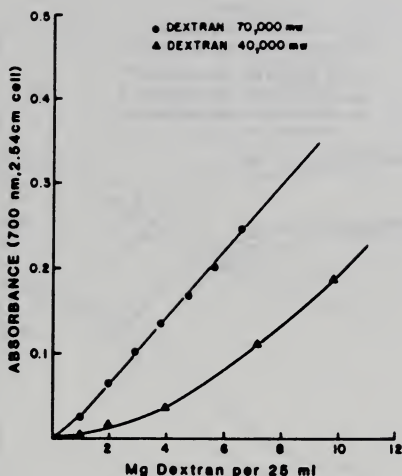


Figure 2.-- Standard curves for dextrans of 40,000 and 70,000 mw. Add absolute alcohol 1:1 by actual measuring, stand 20 min.

Materials and Methods

Samples of fresh raw juice (crusher juice) were provided from the W. R. Cowley Sugar House, Santa Rosa, Texas. Each sample was divided into two equal portions. One portion received no additive, while another portion received 20 ppm (by weight of juice) of a biocide (Olin 3302).

The two portions were then kept in a constant temperature water-bath at 30°C, with occasional stirring. Aliquots of each portion were withdrawn after 0, 2, 4 and 6 hours for haze determination, and gravimetric analysis for dry solids (DS), sucrose by total inversion, invert, and sulfated ash.

The 6-hour cycle was chosen to simulate a factory where good housekeeping was maintained with mechanical cleaning once every 8-hour shift. The effect of biocide can be shown in juice simulating that trapped inside the milling tandem between cleanings.

RESULTS AND DISCUSSION

Results of gravimetric determinations of sucrose, invert and ash on juice samples were all based on % dry solids. Sucrose and total sugars (as invert) were calculated as ratios to ash. Since ash in the same juice remained unchanged with or without treatment, the changes (loss) of sucrose and total sugars became the only variables. The following figures 3, 4, 5 and

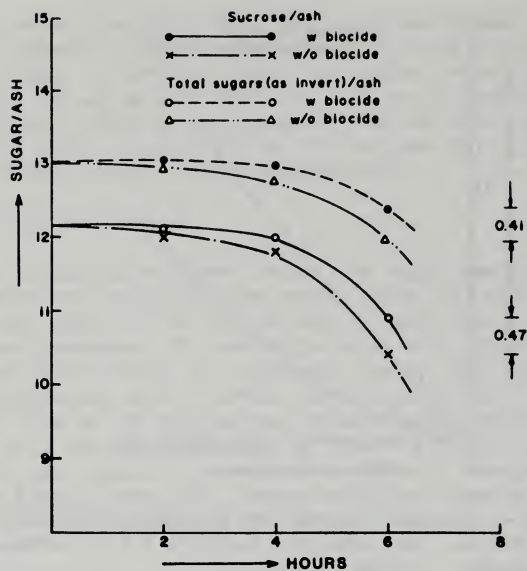


Figure 3.--Effect of biocide on sugar loss.

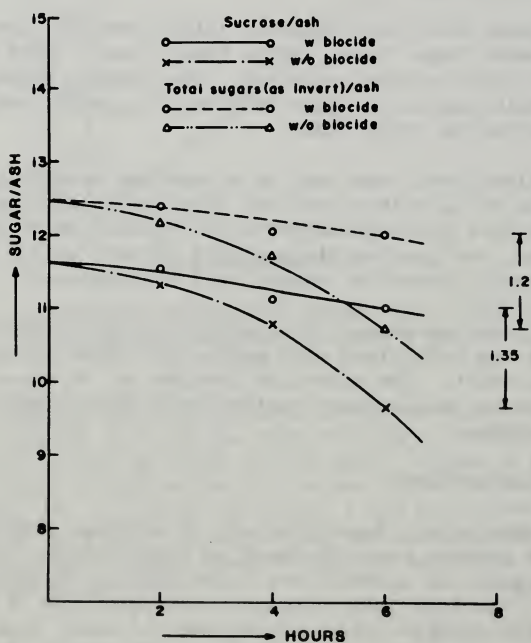


Figure 4.-- Effect of biocide on sugar loss.

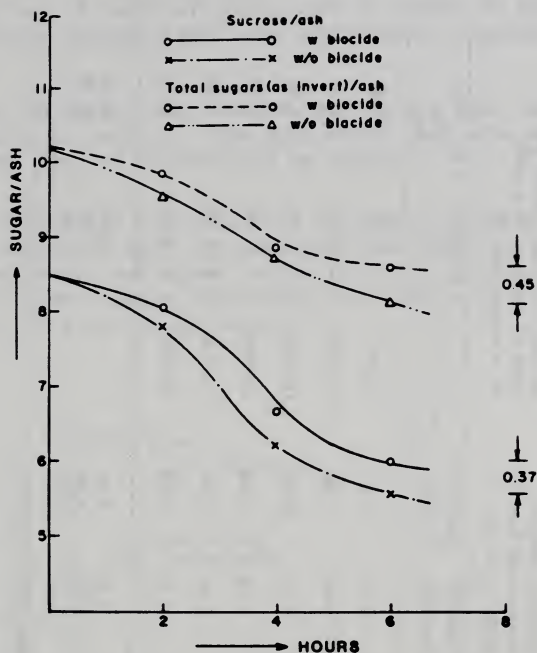


Figure 5.--Effect of biocide on sugar loss.

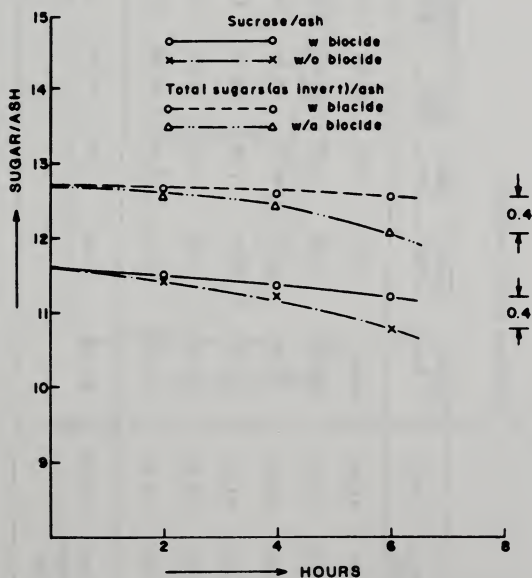


Figure 6.--Effect of biocide on sugar loss.

Table 1.—Gravimetric Analyses of Sugars in Juices before and after biocide treatment.

Date 1981	Original Juice Analyses				Without Biocide Minus with Biocide after 6 hours.		On 1000 T. Cane, losses per 1000 T. Juice x DS x Ash ^z DS = weight Ash (T)		
	DS%	Sucrose ZDS	Invert ZDS	Ash ZDS	Suc. Ash	Total S. Ash	Loss of Suc/Ash	Loss of T.S./Ash	Wt. T.S. (T) ***
4/9	16.85	86.01	1.72	7.06	12.18	13.04	0.47	0.41	4.88
4/14	17.21	85.98	1.86	7.39	11.63	12.47	1.35	1.28	16.28
4/21	14.37	74.29	8.84	8.73	8.51	10.20	0.37	0.45	5.65
4/23	17.32	83.52	2.39	7.97	10.48	11.30	0.75	0.38	5.25
5/19	15.30	83.63	3.60	7.20	11.62	12.07	0.43	0.45	4.96
5/21	14.63	76.13	9.16	7.40	10.29	12.04	0.19	0.25	2.71
Ave.	15.95	81.59	4.60	7.63	10.69	11.83	0.59	0.54	6.62
High							1.35	1.28	16.28
Low							0.19	0.25	2.06

* Total Sugars as Inverts = (Suc. x 1.05) + Invert.

** Wt. Sucrose = wt. Ash x diff. of Suc./Ash ratio, between w. and wo biocide.

*** Wt. Total Sugars as Inverts = wt. Ash x diff. of T.S./Ash ratio, between w. and wo. biocide.

6, show series of tests of sugar loss or change in sugar concentration, against time, with and without biocide treatment.

Then, from the analytical results, the losses were calculated and tabulated in table 1. Two additional test sets appear in table 1 that are not represented as figures.

Next, the absorbance vs. time were plotted on treated and untreated juices in each test series, with also the changing of pH. The curves are shown in the following charts (figures 7 to 10). These figures relate dextran formation, as absorbance, to time and pH change.

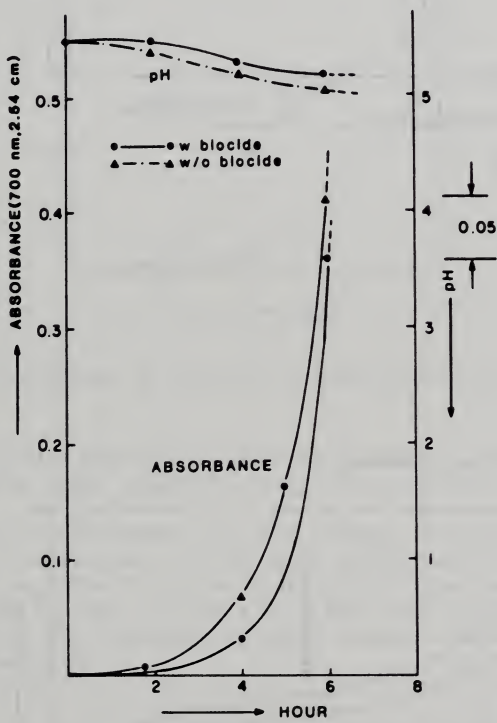


Figure 7.--Effect of biocide on deterioration of sugars.

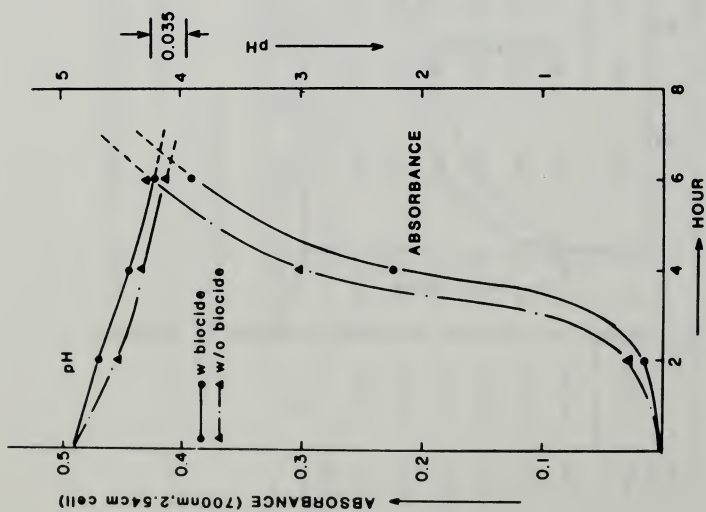


Figure 8.--Effect of biocide on deterioration of sugars.

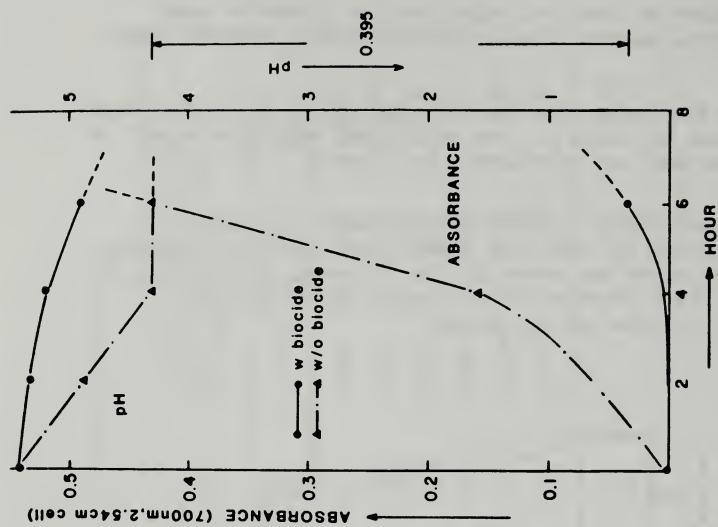


Figure 9.--Effect of biocide on deterioration of sugars.

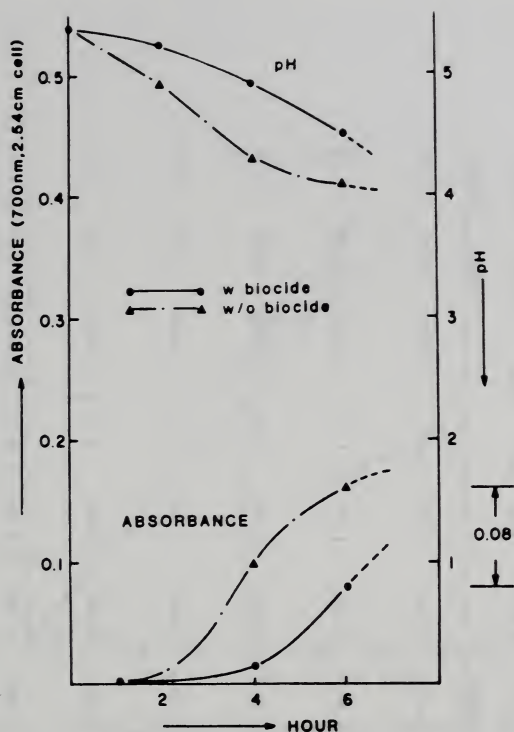


Figure 10.--Effect of biocide on deterioration of sugars.

Absorbances with and without biocide treatment, and corresponding sugar analyses, are shown in table 2.

Differences in absorbance with and without biocide, after 6 hours, were calculated, to represent differences in dextran concentration. There are correlated with sugar losses in table 3; and plotted in figure 11. The sugar losses are calculated on the estimates of total sugars with and without biocide treatment.

Table 2. Absorbance Readings (after 6 hours) vs. Sugar Losses.

Date	With Biocide						Without Biocide					
	Absorb- ance (6 hr)	Loss Suc/ Ash	Loss T.S./ Ash	Ash(T) 1000 T Cane	Suc. Loss (T)/ 1000C.	T.S. Loss (T)/ 1000C.	Absorb- ance (6 hr)	Loss Suc/ Ash	Loss T.S./ Cane	Ash(T)/ 1000 T Cane	Suc. Loss (T)/ 1000C.	T. S. Loss (T)/ 1000C.
1981												
4/9	0.36	1.28	0.66	11.8961	15.23	7.85	0.41	1.75	1.07	11.8961	20.82	12.73
4/14	0.05	0.62	0.47	12.7182	7.89	5.98	0.35	1.97	1.75	12.7182	25.02	22.26
4/21	0.39	2.49	1.62	12.5450	31.24	20.32	0.425	2.86	2.07	12.5450	35.88	25.97
4/23	0.035	0.81	0.72	13.8040	11.18	9.94	0.43	1.56	1.10	13.8040	21.53	15.18
5/19	0.08	0.40	0.16	11.0160	4.41	1.76	0.16	0.83	0.61	11.0160	9.14	6.72
5/21	0.58	0.33	0.17	10.8262	3.57	1.84	1.40	0.52	0.42	10.8262	5.63	4.55
Ave.	0.25				12.25	7.95	0.53				19.68	14.57

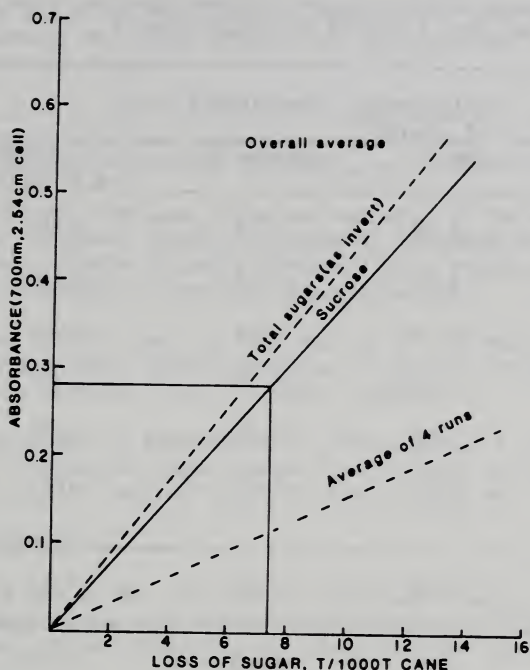


Figure 11.--Difference in absorbance after 6 hr vs sugar losses, with and without biocide.

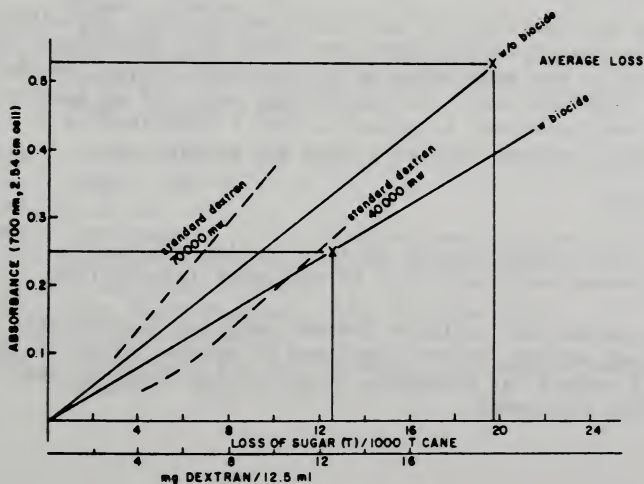


Figure 12.--Absorbance after 6 hr vs sugar loss.

Table 3.--Difference of absorbance (without biocide minus with biocide after 6 hours) and sugar losses.

Test #	Date 1981	Difference of Absorb- ance	Loss/1000 T Cane	
			Sucrose (T)	Total Sugars (as Inverts (T))
1	4/9	0.05	5.59	4.88
2	4/14	0.30	17.17	16.28
3	4/21	0.035	4.64	5.65
4	4/23	0.395	10.35	5.25
5	5/19	0.08	4.74	4.96
6	5/21	0.82	2.06	2.71
Overall Average		0.28	7.43	6.62
Ave. of * #1,2,3, 5		0.12	8.03	7.94

* Of low absorbance diff.

The plots in figure 12, of absorbance values with and without biocide treatment versus sugar loss show that absorbance values are in the same range as the standard curves for the standard dextrans used. The additional axis, of mg dextran/12.5 ml test solution, is added to show a quantitative relationship between dextran formed and sugars loss.

CONCLUSIONS

1. The Haze method adopted in this testing program can be reproduced satisfactorily in different laboratories.
2. The gravimetric analyses demonstrated the considerable loss of sugars, either expressed as sucrose alone or as total sugars (as inverts), in the milled juice without biocide treatment.

3. There was still some loss of sugars even with biocide treatment, but these losses were approximately half those on untreated juice.
4. The plots of the difference of absorbance with and without treatment, and the respective sugar losses, demonstrate the benefit of biocide treatment, as in figure 12.
5. In milling, the same juice may not stay in the mills for 6 hours, but some juice is frequently retained over that period. Levels of deterioration in the study agreed with those obtained by previous investigations.
6. It was evident that pH drop was not a good yardstick for measuring sugar losses, because it was too irregular and did not correlate well to sugar figures.
7. By relating the absorbance of juices (after 6 hours) to their respective sugar losses, the overall average fell on the standard dextran line of around 40,000 mw.

ACKNOWLEDGEMENTS

Thanks are due to Mr. Jack Nelson, General Manager, W. R. Cowley Sugar House, Rio Grande Valley Sugarcane Growers, Inc., Santa Rosa, TX for providing the factory juice samples.

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DISCUSSION

M. FOWLER (Amstar): I would like to see the exact compound that was used as a biocide. There are 5 or 6 biocides approved for use in cane mills in the U. S.

M. A. CLARKE: The commercial name of the compound appears in the paper; it is a carbamate compound.

JOHN COOPER (Savannah): In the loss figures in tables 1 and 2, the total sugars loss is less than the sucrose loss in some samples. How can this be?

M. A. CLARKE: I think that it is an artifact of the way these measurements were made. Total sugars were measured as invert after hydrolysis by acid. The sucrose was analysed by the Clerget Method, which is a pol measurement before and after acid treatment. In the Clerget, I think that some dextran or oligosaccharides were hydrolyzed, too, giving the illusion of a higher sucrose content than was actually there.

THE CONDITIONING OF REFINED SUGAR IN SOUTH AFRICA

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Sugar Milling Research Institute

INTRODUCTION

The phenomenon of caking or stickiness of raw and refined sugars has long been known to sugar technologists all over the world. However it was not really until the introduction of bulk handling and distribution systems for refined sugar, that this became a serious problem for the producer. During the fifties and sixties, many investigations were conducted into the cause, prevention and measurement of caking in refined sugar. Much of this work is covered in a comprehensive review of the subject by Bagster (1970).

It has now been widely accepted that the cause and mechanism of caking are related to the movement of the residual moisture content in the bulk sugar (Rodgers and Lewis 1962, idem 1963). It has also been established that this phenomenon can be minimised or virtually eliminated by subjecting the bulk sugar to a "curing", "conditioning" or "aging" period, under certain prescribed conditions (Stachenko et al 1966).

Sugar conditioning silos are large and costly installations. Hence before the design of the first bulk handling system in South Africa was finalised, it seemed prudent to undertake a few simple and practical tests to establish whether refined sugar from Hulett's Refinery in Durban conformed with the established behaviour patterns for conditioning. In particular, it was necessary to determine whether the somewhat larger grain size, the relatively wider variations in grain size and the high conglomerate count of this sugar would have any substantial influence on curing behaviour. This paper gives details of the tests performed and the results obtained.

EXPERIMENTAL PROCEDURE

Experimental Conditioning Silos

Two pilot scale silos were constructed of stainless steel as

shown in Figure 1. Each silo was 250 mm in diameter, 1000 mm high and had a capacity to hold 40 kg of sugar, supported on a fine wire mesh screen. The contents of each silo were maintained at a constant and uniform temperature by controlled heating of the vessel walls, which were wrapped externally with silicone rubber heating tapes. A voltage of 40–60V was required to maintain temperature equilibrium. Each silo was also provided with a thermometer in the centre and an opening at the bottom for sampling.

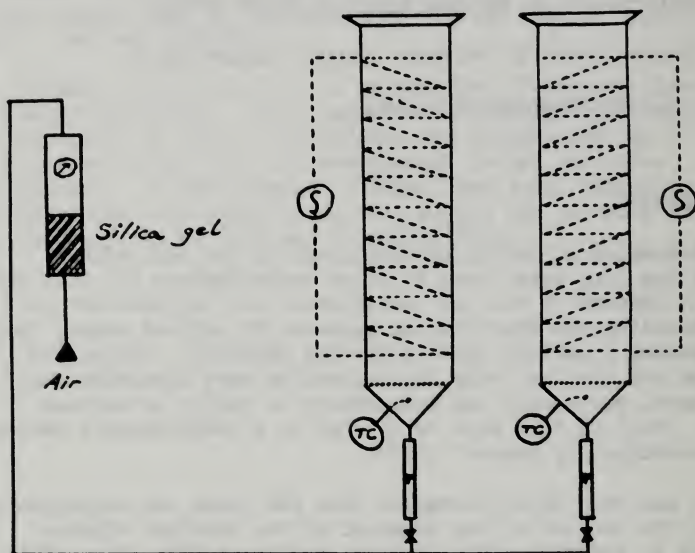


Figure 1.—Schematic diagram of the pilot conditioning silos.

Compressed air at 20% relative humidity (RH) and preheated to the required temperature by means of a tungsten bulb, was introduced at the bottom of each silo. A thermocouple and proportional temperature controller were used to maintain the correct air temperature. The air was pre-dried to below 20% RH with silica gel. Humidity was measured by means of a calibrated hair hygrometer and controlled at the desired value by bleeding a small amount of moist air into the pre-dried stream. Air flowrate were regulated with small rotameters.

The air flow rate was controlled at 2 l/minute per silo (equivalent to 50 l/min/1000 kg sugar), which is a typical figure used for many commercial installations. However it should be noted that, as a result of scaling down, this produces a superficial air velocity in the silo of only 4 cm/minute, which is considerably lower than for industrial scale installations.

Miniature silos consisting of glass test-tubes (23 mm x 200 mm) were used for certain small scale experiments. Their temperature was controlled by immersion in a thermostatic water bath. Air entering these silos was warmed by passage through 3 m of coiled copper tubing immersed in the same water bath. The air flow rate to each miniature silo was not individually controlled, but the overall flow to the distributor manifold was regulated. Silica gel was used, as previously described, for humidity control.

Caking Tests

With the proposed bulk handling system, conditioned sugar is transported to Johannesburg (600 km inland from Durban) by rail. The specially designed tankcars have a diameter of some 3 m and a nominal capacity of 50 mt. It was suspected that the most likely place for caking to occur would be during transit, because of the considerable variations between day and night temperatures, especially in the inland areas (sometimes greater than 30°C).

To establish the potential caking characteristics in transit, it was decided to construct a suitable small scale simulator of a rail tankcar, as shown in Figure 2.

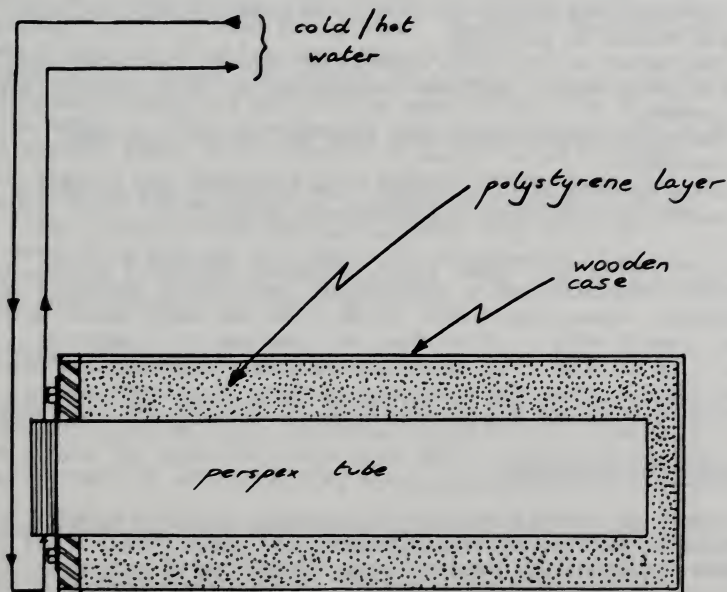


Figure 2.—Schematic sketch of the railcar caking simulator.

This comprised a 200 mm diameter perspex tube sealed at one end. All sides, except the open end, were well insulated by a 50 mm polystyrene layer and the whole assembly mounted in a wooden box. The open end was fitted with an airtight metal lid through which water could be circulated. Thermocouples were mounted near to each end of the tube.

This simulator, which held about 40 kg of sugar, was 1400 mm in length, which corresponds to half the diameter of the proposed rail tankcars. The intention was that the sugar at the closed end of the tube would represent the material in the centre of the railcar, whilst that in contact with the metal lid would be the sugar at the outer wall of the tanker. By adjusting the temperature of the water circulating through the hollow lid, it was possible to subject the sugar in the simulator to a severe set of temperature gradients.

Details of the test procedure are as follows:-

- (a) Before filling the simulator, the air in the tube was dried for 24 hours under tightly sealed conditions using silica gel.
- (b) The silica gel was removed and the hot, conditioned sugar was transferred quickly from the conditioning silos to the simulator.
- (c) The sugar was packed as tightly as possible and the metal lid sealed in position.
- (d) Cold water at 2-5°C was circulated through the hollow lid for 16-20 hours, followed immediately by water at 60°C for 6 hours.
- (e) The sugar temperatures near each end of the tube were recorded.
- (f) After approximately 24 hours, the simulator was carefully opened and the sugar inspected for caking.

In later tests, this large scale simulator was found to be somewhat cumbersome and a simplified, small scale procedure was developed. Glass test-tubes (28 mm x 200 mm) were filled with sugar, sealed with a rubber bung and immersed to a depth of about 5 mm in cold (3°C) water for 20 hours, then hot (65°C) water for 6 hours. The results from these "test-tube caking testers" correlated fairly well with the large scale simulator.

Moisture Determination

The theory of the distribution of moisture in white sugar crystals is well established (Rodgers and Lewis 1963). Most authors differentiate between three categories of moisture:

Free moisture. All the individual crystals coming from the centrifugals are surrounded by a more or less dilute sugar solution. A very large proportion of the moisture present in this film is relatively easy to remove in conventional driers.

Bound moisture. This is moisture which is on the surface of the crystal but which is comparatively difficult to remove in that it takes time. Rodgers and Lewis (1963) are of the opinion that this bound moisture is the greatest enemy to bulk handling.

Inherent moisture. This is moisture which is actually trapped within the crystal and is only determined analytically by grinding or dissolving the crystal. Although there is no direct evidence of the migration or release of this inherent moisture in troublesome amounts, even over prolonged periods of storage (Rodgers and Lewis 1963), Powers (1954) has noted that there is more included water with larger crystals. In view of the large grain size and high conglomerate counts for local refined sugar it was decided to adopt an analytical method for moisture which would determine the total water content of the sugar.

The moisture content of the sugar samples was determined by an automatic Karl Fischer titrator (Metrohm) using the formamide method. The Karl Fischer reagent was standardised against oxalic acid dihydrate which was stored over saturated potassium bromide in a desiccator. Details of the analytical procedure are as follows:

- (a) 60 ml of formamide was added in the titration vessel and automatically titrated to dryness. Manually the read-out was brought to 95 which took about 1 hour.
- (b) About 20 g of sugar was weighed in a test-tube to exclude atmospheric moisture and quickly emptied into the titration vessel. The test-tube was then reweighed and the mass difference taken as the amount of sample being titrated.
- (c) The mixture was stirred for 30 minutes after which the sugar was titrated manually, keeping the indicator at 95.
- (d) A blank titration was carried out with only 60 ml of formamide to measure the uptake of atmospheric moisture during the 30 minute stirring period. The actual titration was then corrected for this small amount.

Conglomerate Testing and Sieve Analysis

Sieve analysis was carried out according to the standard method used in South African sugar factories (Anon 1977). From this analysis data the specific grain size (SGS), mean aperture (MA) and coefficient of variation (CV) were calculated.

Conglomerate counts were based upon the following procedure:

- (a) 100 g of sample were sieved through 850 micron and 425 micron sieves.
- (b) The major fraction on 425 microns, which is about 70% of the sample, was retained.
- (c) This fraction was passed through a 600 micron sieve and

- collected on a 5 cm wide piece of transparent sticky tape.
- (d) The crystals were covered with a similar piece of tape. In this way a slide is produced holding about 1000 crystals.
 - (e) This was put on a photographic enlarger and a print made.
 - (f) A section of 100 crystals was marked out for conglomerate counting. All twinned crystals, clusters or star shaped crystals, and occluded, protruding or adhering crystals were classed as conglomerates.

RESULTS AND DISCUSSION

Influence of Conditioning Temperature

At Huletts Refinery, sugar leaves the granulators at 42-47°C under normal conditions. Hence a number of conditioning tests were undertaken at 40°C and 50°C in order to establish whether temperature had any marked influence over this range.

The table below summarises all our results for the comparison of conditioning at these two temperatures.

	<u>40°C</u>	<u>50°C</u>
Number of trials	10	4
Mean moisture loss after 48h	0,029%	0,034%
Mean moisture content after 96h	0,051%	0,040%

During the initial 48 hours the mean rate of drying was greater at 50°C than at 40°C, but the difference is not statistically significant. This difference would probably become significant if more trials at 50°C were included.

Even with the limited number of trials, the moisture level after 4 days was significantly lower at the higher temperature, indicating that lower moisture contents are achieved more rapidly at higher temperatures.

Stachenko et al (1966) have shown distinct temperature effects with higher temperatures giving both increased rates of drying and lower final moisture contents. This is supported by the data shown in Figure 4 where conditioning temperatures were raised to 70°C for about 10 hours and then dropped again. This produced an immediate increase in the subsequent rate of moisture loss, particularly for the larger grain sizes.

Bagster (1970) mentions, however, that the rates of drying at different temperatures become equal after the first day and he suggests there is no advantage in using heat for longer than this period.

Effect of Sugar Dust on Conditioning and Caking

To investigate the effect of sugar dust on conditioning and

caking it was necessary to de-dust 40 kg of sugar without cooling or drying it. In an attempt to do this, the sugar was shaken manually on a 100 mesh (150 micron aperture) screen in the hot room (38°C) at the refinery. Dust equivalent to 0.3% of the total sample was collected below the screen, whilst some finer dust escaped to the atmosphere. Unexpectedly this de-dusted sugar absorbed moisture during the screening process, so a meaningful comparison of the conditioning rate of this material with its (unscreened) control sample was not possible. However there were no obvious differences between the caking characteristics or final moisture contents of these two sugars.

For further investigations of particle size effects, the test-tube conditioning silos were developed. These required only 55 g of sugar so it was possible to experiment with small samples collected in a sieve-analysis apparatus. Particles collected in the bottom pan (less than 355 microns) were considered as dust and it was then possible to compare the rates of conditioning of dust alone, de-dusted sugar and the original sample (unscreened). The results are given in Figure 3 and show that dust carried less moisture per unit mass than either the de-dusted or the original sugar samples. Furthermore the dust attained a stable moisture content more rapidly than did the larger particles. Thus there are no indications that dust interferes with sugar conditioning. A test-tube caking test on partially conditioned sugar showed that de-dusted sugar caked at least as extensively as normal sugar.

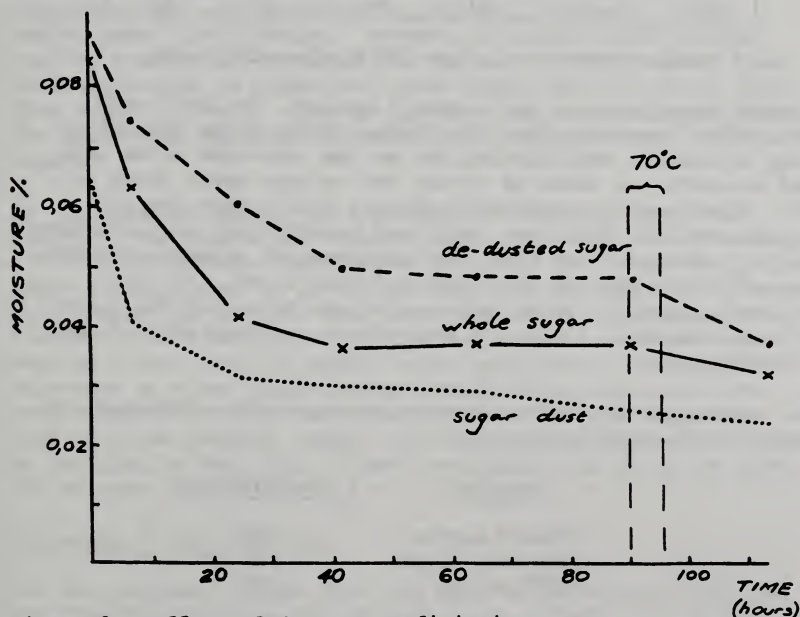


Figure 3.—Effect of dust on conditioning.

These results suggest that there is no need to de-dust sugar prior to conditioning and bulk transport. Farag (1979) also indicated that dust does not contribute to caking. He found that sugar dried at 145°C formed more dust but less cake than sugar dried at 100°C.

Effect of Various Grain Sizes on Conditioning

One batch of sugar caked in all caking tests, even after 168 hours of conditioning. Sieve analysis revealed that this sugar had an unusually high percentage of large (greater than 1700 micron) grains and a high coefficient of variation. This is illustrated in Table 1.

Table 1.—Sieve analyses for various sugars used

Aperture (microns)	Mass percent retained				
	A	B	C	D*	E
1700	0,05	0,1	0	0,5	0,2
1180	0,2	0,3	0,3	0,8	0,6
1000	0,6	0,8	1,3	0,7	1,9
600	39,0	33,1	36,1	31,2	42,3
355	42,9	48,8	42,4	53,9	43,8
< 355	17,2	16,9	19,7	13,0	11,2
MA mm	0,469	0,500	0,525	0,465	0,565
CV	43	51	46	67	45

* Sample caked even after 168 h of conditioning.

The conglomerate count for sample D was not, in fact, very high. This suggested that the larger grains might prevent or delay successful conditioning and so the test-tube silos were used to study the rates of drying for various grain sizes at 40°C. The results presented in Figure 4 show that the two largest particle fractions contained considerably more moisture than any other cut and that they continued to release moisture long after the smaller fractions had stabilised. It is noteworthy that the sugar which could not be successfully conditioned was still releasing appreciable moisture after 7 days of conditioning.

The results of conglomerate counts on the various fractions of sample D are shown below:

<u>Fraction</u>	<u>% Conglomerates</u>
355-600 micron	86
600-1000 micron	100
>1000 micron	100

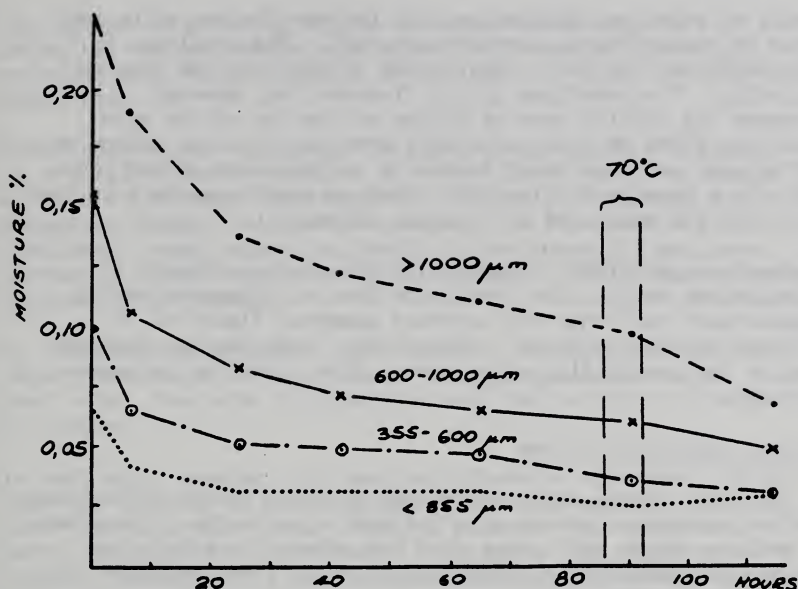


Figure 4.—Effect of grain size on conditioning.

These results emphasise that the larger particles (mainly conglomerates) are important determinants of drying rates during sugar conditioning. Rodgers and Lewis (1963) showed similar results and according to Chapman (1970) it takes about 12 hours longer to condition coarse sugar (MA 0,5-0,6 mm) than fine sugar (0,3 mm). This is also confirmed by McGimpsey (1960) who found that the time to reach equilibrium was less for finer sugars.

In the standard method for conglomerate counting (described earlier), the counts are done on sugar which has passed through a 600 micron screen. The results presented have shown that, for sugar conditioning, it is the particles larger than 600 microns which cause the problems and hence the standard conglomerate counts may be meaningless because they reflect a measurement made on a size fraction which is not problematic. Perhaps for relevance to sugar conditioning specifically, the method for conglomerate counting should be modified. It is suggested that only the genuine, large, star-shaped conglomerates should be included in the count. These predominate in particles above 600 microns.

Influence of Air Flowrate

Air was purposely leaked from the base of one silo so that the air flow to that silo was reduced almost to zero. An 80 kg

sample of sugar was divided equally between the two silos and rates of conditioning were then compared. The results indicated that, in fact, very little air is required for conditioning. The reduction in air flowrate did, however, decrease the initial rate of drying at the top of the silo. After the first 48 hours, air flow apparently had no effect on drying rate and even sugar stored in an insulated polystyrene box with a loosely fitting lid, dried as rapidly as that in the silo with 2 l/minute of air passing through it.

Stachenko et al (1966), Chapman (1970) and theoretical calculations support the conclusion that air flowrate can be considerably less than the commonly accepted figure of 50 l/minute per ton of sugar. However the lower the air rate the greater the possibility of an uneven flow distribution across the silo.

Optimum Conditioning Time

In practical terms, sugar conditioning should aim to reduce the residual moisture content of a refined sugar to the point where no serious caking will occur when the sugar is subjected to temperature gradients slightly in excess of those expected in the real situation.

Rodgers and Lewis (1963), in summarising U.S. practice, quoted residence times of 24 hours, which they suggest is too short a time. Many commercial installations aim at 36-48 hours.

In the present study, drying curves indicated that moisture removal was still substantial after 48 hours. In most cases it required in the region of 72 hours before the drying curves levelled off at residual moisture contents of 0,045-0,050%. Subsequent caking tests, in which these sugars were subjected to fairly severe thermal gradients (as previously described), indicated that in most cases a 72 hour conditioning period would be adequate. It is suggested that the relatively large grain size, coupled with the high conglomerate levels, are the cause for this longer curing period.

Potential Changes in Residual Moisture

If the residual moisture in conditioned sugar (largely inherent moisture) was slowly released under prolonged storage, it could cause caking by migrating to the coolest areas. To test this danger, conditioned sugar from the 40 kg silos was stored for six weeks in tightly sealed plastic buckets and then returned to the caking simulator. In no case (4 tests) did sugar cake after storage if it had not caked before storage. The one sample which had caked prior to storage, did so to a lesser extent after the six weeks storage.

Another potential problem is that of moisture regain after con-

ditioning. A series of simple tests was undertaken to establish whether conditioned sugar could absorb appreciable moisture when briefly exposed to ambient air during loading operations.

Conditioned sugar was spread in a petri dish which was closed in a balance chamber together with wet filter paper to create a moist atmosphere. The sugar was weighed periodically to determine changes in moisture content. It was found that stationary sugar exposed to humid air increases its moisture content at 0,01% in 10 minutes (1 mm layer) or 0,0076% in 10 minutes (10 mm layer).

To simulate the situation where sugar is moving in a screw conveyor, humidified air was passed through conditioned sugar in a test-tube. The rate of absorption was then as high as 0,03% in 10 minutes.

In each case, however, the absorbed moisture evaporated rapidly when the humidity of the surrounding air was decreased and the sugar formed a hard cake. Thus, if moisture is re-absorbed during loading it is potentially very mobile and could cause caking. Moisture will not, however, be absorbed unless the RH of the surrounding air exceeds the so-called equilibrium relative humidity (ERH) of the sugar.

Rodgers and Lewis (1962) state that it is dangerous to allow an RH greater than 65% in the air surrounding the sugar. McGimpsey (1960) quotes ERH values between 68 and 75% for granulated refined sugar.

Conditioned sugar is normally warm (~35°C) when loaded. This would heat the surrounding air in contact with the sugar, thereby decreasing its RH. It was noted that for air to exceed an RH of 70% at 37°C (i.e. to pose a potential danger for re-absorption), the initial air would need to be saturated (RH = 100%) at 30°C. Thus it seems unnecessary to take special precautions to protect warm sugar against moisture re-absorption from ambient air during loading.

DESIGN OF FULL SCALE INSTALLATION

Based upon the data from the present study, together with the considerable body of published information, a full scale installation was erected and commissioned at Hulett's Refinery in Durban during 1981.

The silo is constructed of steel and has a nominal working capacity of 750 mt. It is 8 m in diameter and 30 m high. Conditioning air (35 m³/minute) is supplied at 42°C and 20% RH. Initial operating results have indicated that the residual moisture contents of conditioned sugar from the full scale installation are slightly lower than was obtained in the pilot

scale tests. No serious caking problems have as yet been encountered during the warm summer period. However the system is not yet operating at design throughput rates nor has the conditioned material been subjected to the more severe temperature gradients encountered during winter. These results are anticipated with great interest.

CONCLUSIONS

Results from this investigation indicate that, despite the high conglomerate count and coarse grain size of Hulett's refined sugar, it can generally be conditioned adequately in 72 hours. Problems may be encountered with sugars containing a high CV and an exceptionally high percentage of crystals larger than 1700 microns. These parameters may prove useful indicators of sugars which would be difficult to condition. The standard conglomerate counting procedure is of dubious value in this regard.

The conditioned sugars generally contained 0.04 to 0.05% moisture as determined by the Karl Fischer analysis using formamide to dissolve the sugar. Conditioned sugars from other countries are reported to contain as little as 0.01% moisture but this can be misleading because different methods for moisture determination are often used and these are known to give different results. Moroz et al (1967) found that sugar conditioned for 72 hours contained 0.036% moisture by the Karl Fischer-formamide method but only 0.012% when determined by standard oven drying (105°C for 3 hours).

The alternative Karl Fischer method using anhydrous methanol to extract the moisture is expected to give lower results than formamide because methanol does not dissolve the sugar, thereby releasing the inherent (trapped) moisture. Stachenko et al (1966) present results which indicate that the Karl Fischer-methanol method gives results very similar to oven drying.

Sugar dust conditions more rapidly than whole sugar, whilst the influence of grain size on the rate of moisture release has been demonstrated clearly. Moisture re-absorption by conditioned sugars is potentially a rapid process, but is unlikely to occur during the loading of warm sugar because the RH of air in contact with the sugar will seldom exceed the ERH of the sugar.

The development of test-tube scale conditioning silos and caking testers is an advance which can facilitate future studies. These test-tube silos gave results which were similar to the 40 kg pilot silos and have the advantage that moisture changes can be monitored by simple weighing instead of the time-consuming Karl Fischer analyses. Because they require only small quantities of sugar, a large number of conditioning tests can be run simultaneously in the same water bath.

The test-tube caking test proved a more reliable indicator than the large railcar simulator, which proved difficult to seal. It is simple to use and could prove suitable for routine checking of the caking potential of sugars.

ACKNOWLEDGEMENTS

The authors are indebted to Hulett's Refinery Limited, the S.A. Sugar Association and to Bosch and Associates for permission to publish this data and for their assistance in the preparation of this paper.

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DISCUSSION

E. D. STEPHENSON (Atlantic): You referred in your paper to discovering that the amount of air circulation was perhaps not very important. In your final design did you use that, or did you come to some optimum amount of air for clean sweeping of the silo? Are you using recirculation of the sugar at all, or is it a static silo with only movement of conditioning air?

A. B. RAVNO: As far as air flow goes, it was designed on a rate of 50 liters per minute per ton of sugar to be conditioned. This figure is one which we took from the literature and has been mentioned by several people such as Stachenko and Chapman. In our case this works out to a total air flow of around 35 cubic meters per minute. The design is for continuous plug flow operation. Sugar is loaded continuously at the top, has approximately three days retention, and is removed continuously at the bottom for railcar loading. During periods when no fresh, unconditioned sugar is entering the top of the silo, the contents of the silo are kept in motion by recycling from the bottom back to the top until all the contents have completed the 72 hour conditioning period. Thereafter the sugar can be left standing for several days or even weeks without adverse effects.

S. E. GEORGE (BC Sugars): You spoke of conglomerates made in the pan. What type of granulators do you have? Is it a conventional Hershey type granulator where the sugar is lifted and drops down, or is it the Roto-louvre where the sugar rolls around in the bottom? The reason I ask is because in Vancouver we boil a good grain but by the time it goes through the Roto-louvres it comes out what we call agglomerated as distinct from conglomerated. But, the result is the same; it is like popcorn. However, by the time it goes through the conditioning silo, which is one of two storage silos in series, these agglomerates are essentially all broken up.

A. B. RAVNO: In all honesty I must say that I have not looked at the problem of agglomeration in the driers themselves. Our granulators are of the Hershey cascade type, but I have not looked at conglomerate counts before and after these units. It is quite possible that the high moisture levels which we found before conditioning could have been due to both residual surface and bound moisture and to trapped moisture as a result of this weak agglomeration in the driers. However, we still have fairly high moisture levels of around 0.05% in our conditioned sugar and this is certainly due largely to inherent moisture which is trapped within genuine conglomerate crystals which do not break up during conditioning.

S. E. GEORGE: What method did you use to measure moisture?

A. B. RAVNO: We opted to use the Karl Fischer using formamide as the solvent. This solvent dissolves the whole sugar and we measure the total moisture. When we first started we found very low moisture contents in the literature and very high moisture contents in South Africa. We guessed that it was because we were using a method that measured even the water inside the crystal. When we used methanol as the solvent in Karl Fischer we got much lower moisture contents.

S. E. GEORGE: Did you read the paper we gave at SIT, which is an explanation of what we went through in Vancouver roughly 20 years ago? We got out of a very serious difficulty by converting a storage silo to a combination storage and conditioning silo. This worked very well for us.

C. C. CHOU: Have you analyzed your data based on the Kelvin equation of capillary theory? I may publish a paper on this aspect in the near future.

A. B. RAVNO: The answer is No! We set out to do some very simple experiments, just to verify whether conventional practice would apply to our sugars. We came up with sufficient answers to satisfy ourselves. It was a relatively limited set of tests. However, I will look forward to reading your paper.

S. E. GEORGE: I was extremely pleased to hear someone say the opposite of the old wife's tale that the way to make sugar free flowing was to cool it. I have always suggested that in that case take it right out of the centrifugals and put it in a freezer. That will cool it, but it won't make it free flowing. Dr. Ravno has given the true story.

PROCESSING OF UNCONVENTIONAL CANE

Stephen J. Clarke, Mike J. Giamalva, Louisiana State University and David H. West, Private Consultant

Sugarcane has traditionally been grown for processing to sugar, the bagasse being used as fuel for this process and molasses production being minimized. Recent developments in large scale fuel alcohol production utilize the soluble carbohydrates for fermentation and the fiber as process fuel. In both cases the optimum level of fiber in the cane is that required to provide sufficient fuel for process.

Sugarcane varieties are usually selected on the basis of low fiber levels and high juice purity and sugar recovery. Varieties are now being selected for total sugars as feedstock for alcohol plants (Humbert, 1980). Unconventional high fiber varieties with a different balance of components offer the possibility of fiber production as well as sugar and/or alcohol. Such varieties can usually be grown at higher yields and may be more disease and pest resistant.

Fuel requirements for processing may be expressed simply in terms of fiber content or in terms of the ratio of soluble solids (for processing) to fiber (for fuel). Data from Hugot may be used to calculate the minimum fiber requirements for processes with different evaporation schemes, the largest use of steam in process (Hugot, 1972). For triple effect evaporation with no vapor bleeding, a minimum of 12% fiber is required, for quadruple effect with full vapor bleeding, 10%, and for a quintuple effect with full vapor bleeding and thermal recompression, 9%. Processing of cane with higher levels of fiber should result in surplus bagasse for other uses. Conventional cane varieties have a soluble solid to fiber ratio between 1.2 and 1.4. High fiber canes show a much lower ratio and the possibility of using only a part of the fiber or fuel for processing to sugar or processing only part of the cane, that richest in sugar. This could be achieved if the cane could be fractionated into fiber components, the better material being used for by-products and the lower quality material for fuel.

Variety Selection

The varieties studied in this program were originally selected in a search for new varieties with cold tolerance for commercial exploitation in Louisiana. The desirable characteristics of the old noble canes (*Saccharum Officinorum* L.) include low fiber and high sugar levels and thick stalks but they are so susceptible to disease that they are no longer of commercial value. Hybrids between the noble canes and wild canes (*Saccharum Spontaneum* L.) are now standard and the wild canes contribute the valuable characteristics of disease and pest resistance and ratooning ability. However, they also donate the undesirable characteristics of high fiber levels and low juice purity. The typically thin stalks of these canes is compensated for by the large number of stalks. In selecting for canes with high fiber and yields the juice quality is usually poor and this is found with the cane varieties in this study. The number of stalks and their vigor of growth influence cane tonnage and quality. Vigorous growth with a high number of stalks produces high tonnage and poor juice and these features were found in this study.

New varieties are usually produced by crossing of hybrids and by the introduction of new material into the gene pool. L79-1002 was produced from a commercial hybrid, CP52-68, and a wild cane from Argentina, Tianan 96. L79-1003 was a result of a cross of two hybrids, NCo310 and US61-21.

Yields and Composition

The yields and composition of the cane varieties under study and some standard varieties are listed in Table 1. Gross cane includes tops and trash and net cane is the material that should be delivered to the mill.

CP65-357 is the most common commercial variety in Louisiana and CP70-321 is a similar variety released a few years ago. Both show the typical composition for commercial cane varieties. L79-1002 and L79-1003 are the two varieties used most in this work and US74-31 and L79-33 are two varieties recently added to the study. Analyses for composition were carried out by the press method after complete disintegration of the cane.

The inverse relationship between fiber levels and juice purity is clear from this data. The advantages of these varieties are in the high yields, more than double the standard variety with L79-1002.

Processing Alternatives

Several possibilities present themselves as alternatives for

Table 1. Yields and Composition of Cane Varieties

VARIETY		GROSS CANE PER ACRE	NET CANE PER ACRE	COMPOSITION OF NET CANE			
		(TONS)	(TONS)	FIBER %	SOLUBLE SOLIDS %	POL %	JUICE PURITY
CP65-357	(1980)	40	35	12.8	16.8	14.9	89
	(1981)	38	32	13.0	16.5	14.6	88
L70-321	(1980)	37	31	12.4	16.7	15.0	90
L79-1002	(1980)	82	68	25.5	9.6	6.9	72
	(1981)	97	80	28.0	11.1	8.3	75
L79-1003	(1980)	65	52	16.9	9.6	6.9	72
	(1981)	68	55	17.0	10.8	8.0	74
US74-31	(1981)	NA	NA	17.5	13.9	11.7	84
L79-33	(1981)	48	40	15.4	13.7	11.3	82

the processing of this type of cane. The first stage of the process, juice extraction, is where the different composition of the cane will have the most impact. Juice clarification, evaporation and crystallization would be similar to that for conventional cane, although the quantities of sugar are lower. Processing to alcohol has not been studied extensively although it is a possible use for the juice.

Conventional processing has the advantage of good extraction and that a conventional mill may be used. The main disadvantage is that the throughput of a mill is best expressed in terms of fiber. The throughput of these canes would therefore be much lower than for normal varieties. Standard imbibition on the mill requires 200% water on fiber for good extraction and this would result in considerably diluted juice. The steam balance of the factory would be upset since the live steam requirement for the extraction equipment would not match the exhaust steam necessary for processing the lower amount of juice.

A simple method for extraction involves disintegration of the cane followed by pressing to extract the juice without imbibition. The live steam required for the prime movers in this case is less than for conventional milling and a better steam balance could be achieved. The disadvantage is the poor extraction achieved, especially for high fiber materials. The extraction achieved by pressing depends mainly on the fiber content and the residual moisture levels. Extraction decreases with both increasing fiber and increasing residual moisture. The minimum moisture levels that have been achieved are just below 30% using a screw press and if an extraction level of 90% is considered minimum, then this may be achieved

with material of not more than 15% fiber. Pressing 25% fiber cane to 50% moisture gives extraction of only about 50%. For low fiber content materials this is the best method for extraction. Juice brix has a smaller effect on extraction, the higher the brix the lower the extraction. If the principal use of this type of cane is fuel, then pressing to 50% moisture, the maximum at which combustion may be maintained, may be appropriate.

The Tilby Separator, in which the more fibrous rind is separated from the interior of the cane, is a means of fractionating the cane into components which may be processed separately. The juice obtained by extraction of the pith is usually of higher purity than from the whole cane and the rind is available for other uses. Disadvantages of this process are the loss of sugar in the rind and the usually insufficient fiber in the pith for use as fuel process. This latter problem is overcome by using high fiber cane varieties.

In the following discussion, several assumptions are made for the calculation of sugar yield. Conventional processing means standard milling with 200% imbibition on fiber, 48% moisture in bagasse, last mill juice brix of 4 and 50% fiber in bagasse. Mixed juice purities are assumed to be the same as for press juice and Winter-Carp molasses exhaustion is the basis for sugar recovery. Measurement of reducing sugars to ash ratios for the molasses produced in the laboratory and using these values to calculate final molasses target purities suggest that lower purity molasses than Winter-Carp may be feasible. All of the values for potential sugar yield are based on analytical data from the samples of cane used. Variations of up to 20% are to be expected between samples collected at different times and from different sites.

Processing of L79-1002

This extremely high fiber cane grows in very high yields and was initially thought to be of value as "biomass" for fuel. For this purpose the material needs to be processed to remove moisture to the point at which combustion can be maintained. The extracted juice may be used for sugar and/or alcohol production. Separation of this type of cane is not appropriate since the fiber level from the pith is still very high.

The quantities of sugar available from different processes are listed in Table 2. The excess bagasse is that over and above that required for processing of the juice. By conventional processing the sugar yields per ton of cane are at most half that for regular cane but the yields per acre are higher. The problem of mill capacity is limiting and there is the further problem of bagasse storage. The quantities of bagasse

available from this variety, after processing in a regular sugar mill, would be so high that, taken with the low bulk density of bagasse, storage would be almost impossible. Immediate use of the bagasse or compression into a more storable form would be required.

Product values per ton of cane processed are given in Table 3.

Table 2. Sugar and Bagasse Yields from L79-1002 (1981)

PROCESS	POL EXTRACTION	SUGAR YIELD		EXCESS BAGASSE (%)
		(LB. SUGAR/ TON CANE)	(LB. SUGAR/ ACRE)	
CONVENTIONAL	91	122	11,007	50
SHRED AND PRESS TO 30% MOISTURE	80	102	9,203	60
SHRED AND PRESS TO 40% MOISTURE	68	91	8,210	65
SHRED AND PRESS TO 50% MOISTURE	50	67	6,045	70
CP65-357	96	259	9,338	-

Product values per ton of cane processed are given in Table 3. The product values are relative to each other and are based on the potential yields of sugars, etc. Prices used are sugar at 18¢ per lb.; molasses at 2.5¢ per lb.; fuel alcohol at \$1.70 per gallon and electricity at 3¢ per kwh. No matter which extraction process is used, the sugar (or alcohol) from this type of cane is worth much more than the electric power available from the excess bagasse.

Processing of L79-1003

This and similar high fiber varieties are more akin to regular cane and the most appropriate technology appears to be cane separation. The results of the use of this process are given in Table 4. Between 20 and 30% of the cane is lost as rind. The fiber levels in the pith are high enough (near 9%) for use as fuel in a modern efficient mill and the soluble solids to fiber ratio for the pith approaches that of regular sugarcane. Purities are higher for the juice from the pith than from the whole cane.

Table 3. Product Value per Ton of L79-1002 (1981)

EXTRACTION PROCESS	PRODUCT VALUE (\$)		
	SUGAR (MOLASSES)	FUEL ALCOHOL	ELECTRIC POWER
CONVENTIONAL	25 (4)	26	3
SHRED AND PRESS TO 30% MOISTURE	22 (3)	23	4
SHRED AND PRESS TO 40% MOISTURE	19 (3)	20	5
SHRED AND PRESS TO 50% MOISTURE	15 (2)	16	5
CONVENTIONAL (CP65-357)	50 (3)	35	-

Potential sugar yields are given in Table 5 for several varieties, with and without cane separation. In each case the quantity of sugar available per ton of material processed is higher from the pith than from the whole cane and in one case (US74-31) the yield of sugar per ton of material harvested is higher after separation of the cane, due primarily to the increased juice purity. Sugar yields per acre for this type of process with high fiber cane are similar to those with whole regular cane.

Simple pressing of the pith gives fairly good extraction due to the relatively low fiber content. Separation of the cane followed by extraction with a screw press may be the most suitable method for processing of this type of cane - Table 6. Moisture levels of less than 30% have been achieved with a screw press but the throughput is rather low.

DISCUSSION

High fiber cane varieties can yield as much sugar per acre as conventional cane but standard methods of processing, particularly for extraction, may not be most suitable. Further tests and evaluation of extraction methods, including power requirements, are under way and we hope to have a clearer picture by the end of the next season. Processing of the extracted juice to sugar remains the most profitable and there have been no problems so far in any of the process stages to sugar.

The rapid growth and high yields of these varieties, especially L79-1002, suggest that they may be harvestable

Table 4. Performance of Cane Separator

VARIETY			PRODUCT COMPOSITION			
			FIBER	SOLUBLE	POL	JUICE
		% PITH ON CANE	%	SOLIDS %	%	PURITY
CP65-357	WHOLE	-	13.2	16.2	14.2	88
	PITH	80	7.8	16.9	15.2	90
L79-1003	WHOLE	-	17.1	12.1	9.1	75
	PITH	73	8.8	13.0	10.5	81
US74-31	WHOLE	-	17.5	13.9	11.7	84
	PITH	78	8.1	17.1	15.0	88
L79-33	WHOLE	-	15.4	13.7	11.3	82
	PITH	79	9.6	15.1	12.5	83

Table 5. Sugar Yields from Separator after Conventional Extraction of Pith

VARIETY		SUGAR YIELD		
		LB. SUGAR PER TON MATERIAL PROCESSED	LB. SUGAR PER TON MATERIAL HARVESTED	LB. SUGAR PER ACRE
CP65-357	WHOLE	259	259	9338
	PITH	263	209	7533
L79-1003	WHOLE	149	149	8206
	PITH	185	135	7448
US74-31	WHOLE	206	206	NA
	PITH	279	218	NA
L79-33	WHOLE	198	198	9480
	PITH	224	177	8479

before regular sugarcane varieties mature and may be a means of extending the sugar season. Sugar recovery would be fairly low but a surplus of bagasse would be available for the start of the regular season. We plan to plant the L79-1002 variety at St. James for this type of study next year. Cane is already growing at Breau Bridge for grinding at the end of the 1982 season.

Use for the excess bagasse from the very high fiber canes will depend upon the bagasse composition, principally the levels of

Table 6. Sugar Yields from L79-1003 after Different Extraction Processes

PROCESS	SUGAR YIELD		
	POL EXTRACTION %	LB. SUGAR PER TON MATERIAL PROCESSED	LB. SUGAR PER TON MATERIAL HARVESTED
CANE (CONVENTIONAL)	95	149	149
PITH (CONVENTIONAL)	97	185	135
PRESSING OF PITH TO 30% MOISTURE	94	179	131
PRESSING OF PITH TO 50% MOISTURE	86	164	131

cellulose, hemicellulose and lignin (Paturau, 1969).

Preliminary data suggest fairly low levels of hemicellulose and therefore limited potential for conversion to furfural. The fiber is very brittle and the bagasse is easily fractionated into about equal amounts of pithy and fibrous components using vibrating screens. Either of these fractions would provide sufficient fuel for juice processing, leaving the more valuable components for by-product manufacture.

The study of processing for the modestly high fiber canes (L79-1003, etc.) will concentrate on cane separation. The removal of sugar from the rind is necessary if the fiber is to be used for board, or paper and its extraction by diffusion has been studied. The diffusion gives up to 90% extraction after a long residence time. The quality of the juice is poor and it may only be suitable for fermentation or production of molasses.

Acknowledgements

Hawker-Siddeley Canada, Ltd. provided the cane separator and the project was supported by a grant from the U.S.D.A. (#59-2221-1-2-110-0).

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DISCUSSION

ANON: With economics as they are these days, if you grow cane, and you want to make money, you have to get the maximum amount of sugar because the other products you can get are less valuable. Can I ask why you haven't used other methods of extraction such as diffusion? You are considering canes of high fiber, but there are also canes with a lot of sugars but only a small % sucrose. If you are going for alcohol or fuel, you can get a higher yield of total sugars.

S. J. CLARKE: Diffusion was not considered because we had no equipment for doing it. However, the results would be similar to conventional milling. We would get similar levels of extraction from diffusion as from conventional milling. On the growing of cane for total sugars rather than sucrose alone, this has been discussed by Humbert in Hawaii. They call it energy cane. That is another alternative, but we were just looking at the high fiber cane.

M. A. CLARKE: How do you do your fiber analysis?

S. J. CLARKE: The method we use is the press method. We completely disintegrate the cane, press it, analyse juice and the residual.

M. A. CLARKE: As one possible process, you mentioned a disintegrator followed by pressing. Did you use a screw press or another type?

S. J. CLARKE: The type of press used would depend upon the final level of moisture that you wished. At the Audubon Sugar Institute we have gone down to 38% moisture with conventional milling and can get down to 28% with a screw press. The screw press gives the best extraction, but the power requirements are horrendous.

M. A. CLARKE: You mentioned the situation where excess bagasse is produced and burned to generate electricity. In Hawaii this process is practiced. I wonder if Ken Onna from HSPA would care to make any comments on this.

K. ONNA (HSPA): The approach that we are developing as far as getting fiber is to increase the yield of cane so you can get both more sugar as well as more fiber. There is much work to process trash to increase fiber, but the main approach is still higher yield per acre. In that way we get two things that we want, sugar and fiber.

D. WANKLYN (Redpath): One of the costs in producing sugar is the cane transport from the field. It seems to me that with lower sugar and higher fiber the transport costs per ton of sugar are going to be considerably higher. Have you worked out the economics to a point where this factor is worked in?

S. J. CLARKE: No. Although the transport costs will be higher, we have some evidence that the high fiber canes will lower field costs, fertilizer etc.

Symposium.--New methods of analysis and sugar processing technology

TOTAL POLYSACCHARIDE TEST AND QUANTITATIVE METHOD FOR DEXTRAN ANALYSIS

Richard A. Kitchen

B.C. Sugar

TOTAL POLYSACCHARIDE TEST

This test was based on the colour-forming reaction of sugar-containing substances with phenol and sulfuric acid. The original test was published by Dubois, Giles, Hamilton, Rebers and Smith (Anal Chem 1956, 28, 350-356) for the quantitative detection of sugars on paper chromatograms and in solution. Their method was later modified by Roberts and Friloux (Sugar y Azucar, 1965, 65,66-67), who developed a colourimetric procedure which could be used on solutions of polysaccharides. The polysaccharides in raw juice or molasses samples were separated from sucrose, glucose and fructose by chromatography on carbon-celite columns, using alcohol-water mixtures of increasing alcohol concentration for elution. In later work, Roberts, Clarke, Godshall and Carpenter (Sugar J., 1978 40, 21-23) used dialysis as a means of removing simpler sugars from the polysaccharides, followed by reaction of the polysaccharide portion with the phenol-sulfuric acid reagents. These modifications resulted in a test with which one could detect the total soluble polysaccharides in sucrose-invert solutions.

In mid-1980, the then Cane Sugar Refining Research Group asked for a collaborative evaluation of the total polysaccharide test on five different raw sugar samples. B.C. Sugar, as well as a number of U.S. sugar companies, assisted in this evaluation. At B.C.S., we found that the test required scrupulously clean glassware, washed first in detergent, then in acid, and careful avoidance of any contaminants that could be attacked by, and react with, the phenol-sulfuric acid detecting reagents. Initially, the preparation of the glucose standard curve presented difficulties; the analyses in duplicate showed poor reproducibility and, when repeated, quite often did not improve. This problem was solved by introducing a 5 min

heating period in a boiling water bath immediately after addition of the sulfuric acid. The duplicates showed an immediate increase in reproducibility and the number of repeat analyses dropped off considerably. This additional heating step was incorporated into all our subsequent tests, and I believe has since been used by Sugar Processing Research, too.

Early in 1982, we used the total polysaccharide test to detect the soluble polysaccharides in some B.C. Sugars' pancake syrups, both before and after filtration through a molecular filter having a 10,000 molecular weight cut-off. We found that the polysaccharide concentration after filtration was far too low, and that the missing polymers had not been retained by the membrane. We decided, therefore, to evaluate the polysaccharide test on solutions of known polysaccharides, without the presence of sucrose or invert. The procedure that was followed consisted of five essential steps, as follows:

- Step 1 The solubilization of the polysaccharide in water.
- Step 2 The alcohol precipitation of the polysaccharide at a concentration of 80% ethanol.
- Step 3 The filtration and retention of the polysaccharide on a Millipore filter of 5 or 8 micrometers pore size.
- Step 4 The extraction of the polysaccharides from the filter by boiling water.
- Step 5 The determination of the polysaccharides' concentration by reaction of the aqueous extract with phenol and sulfuric acid, and reference of the colour value at 485 nm to a standard glucose curve.

The intention in this evaluation was to isolate particular steps and to evaluate them individually. This proved difficult to do as most of the steps were interdependent, eg. a problem in Step 2, 3, or 4 could only be detected in Step 5, which could also be considered at fault. The only two steps that could be looked at independently were Step 1, a purely visual interpretation, and Step 5, which could be evaluated by analysis.

The first group of polysaccharides that we examined were samples of starch, their characteristics being as listed below:

- Soluble starch (Fischer Scientific, source not identified)
- Soluble potatoe starch (J.T. Baker)
- Potatoe starch (solubility and supplier not identified)
- Corn starch (from home work-shop)

The first three starch samples were solubilized at concentrations of 0.5 mg/ml, by adding a slurry of each starch in water to a larger volume of boiling water. The resulting solutions were all clear (ie. no signs of opalescence), whereas the sample of corn starch gave a milky suspension which later gelled. The corn starch, therefore was not used in our tests.

Aliquots of the three starch solutions, both before and after molecular filtration, were treated as per Steps 2 to 5. The Step 2 alcohol precipitation sometimes resulted in gelling, while Step 3 Millipore filtration usually produced an opalescent or milky coloured filtrate which did not become clear even after repeated filtrations. Furthermore, the duplicates from the same starch solution often behaved quite differently during the Millipore filtration; one sample filtered quickly, whereas the other did not filter at all (or very, very slowly). The polysaccharide concentrations (by step 5) also were extremely variable for all three starch samples, with values from duplicate analyses not even close to one another. The values obtained on solutions before molecular filtration ranged from 2 to 60 % of the known value, whereas the values after filtration from 1 to 82 %.

An explanation for the odd behavior of the starches was given by Dr. Martin Seidman of A.E. Staley Manufacturing, Decatur, IL. Soluble starches apparently have been acid treated, i.e. partially degraded, and are not completely precipitated by 80 % alcohol, 90 % being recommended. All starches, and in particular corn starches, contain fat and protein, which must be extracted or degraded in order to prevent opalescence or milky coloured solutions. A further problem with the use of starch is due to its 20 - 25% content of the linear glucose polymer, amylose. This polysaccharide easily undergoes retrogradation, and can either gel or crystallize out of solution. Our use of starches for the evaluation of the total polysaccharide test was, therefore, an unfortunate choice.

We next considered the dextrans as test materials, as these polysaccharides contained a high percentage of α -1,6 linkages and were, therefore, more water soluble. The polymers examined were:

Dextran T 40	(Pharmacia, molecular weight 40,000)
Dextran 110,000	(Fluka, molecular weight 110,000)
Dextran T 2000	(Pharmacia, molecular weight 2,000,000)

Each of these three polymers was dissolved in water and the clear solutions analyzed by the alcohol precipitation, filtration, extraction and phenol-sulfuric acid reaction sequence, i.e. Steps 2 to 5. The colours were then determined at 485 nm, and the mg glucose/ml of solution corresponding to the colour reading determined from a glucose standard graph. The results for these polysaccharides were excellent, with 97 to 99% of the dextrans being detected. This indicated that the five steps in the total polysaccharide test were nearly quantitative; furthermore, that the treatment by the phenol-acid reagents, followed by the 5 min heating period, was almost completely effective in hydrolyzing the α -(1 \rightarrow 6), α -(1 \rightarrow 3), α -(1 \rightarrow 2) and α -(1 \rightarrow 4) linkages which occur in the dextrans.

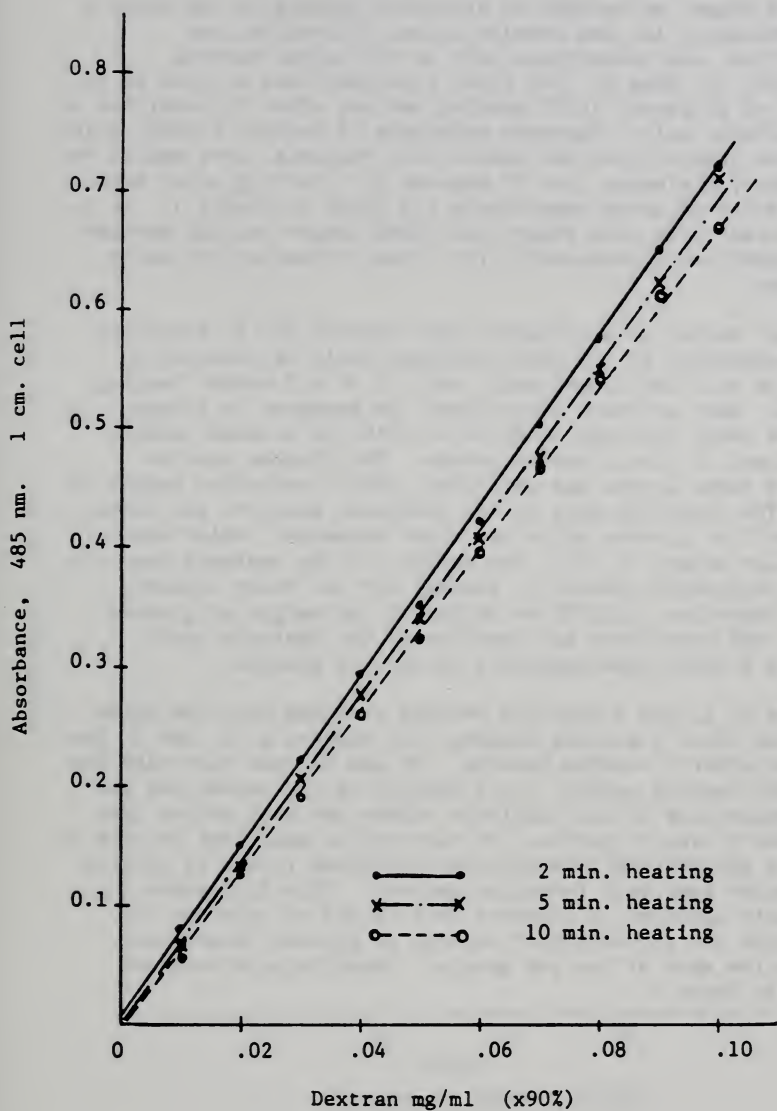


Figure 1.--Effect of different heating times on dextran T 2000 calibration curve.

At this stage, we decided to eliminate testing by the Steps 2 to 4 sequence, ie. the precipitation, filtration, and extraction, and concentrate only on the colour forming reaction, ie. Step 5. Our first experiment was to look at the effect of different 100°C heating periods after the addition of the sulfuric acid. Separate solutions of dextran T 2000, which had been treated with the phenol-acid reagents, were heated for 2 minutes, 5 minutes, and 10 minutes in a boiling water bath. The results of these experiments are shown in Figure 1. It is quite clear from this figure that with longer heating periods the constituents responsible for colour formation are being degraded.

The next series of experiments were carried out to determine what percentage of the three dextrans could be detected by reaction with the phenol-acid, and a 2 or a 5 minute heating period. Each of these experiments was compared to glucose reacted under the same conditions, with the glucose results being used to plot standard curves. The glucose used to prepare these graphs was anhydrous, with a molecular weight of 180. The repeating unit in the dextrans, however, was anhydro-glucose (ie. glucose minus one water molecule), which has a molecular weight of 162. The weights of the dextrans used (ie. after adjustment upwards to account for the water content) were, therefore, 162/180 or 90.00% of the weight of glucose; thus, 100% hydrolysis and reaction of the dextrans would produce a curve superimposable on that of glucose.

Figures 2, 3, and 4 show the results obtained with the three dextrans after 5 minutes heating, and figures 5, 6, and 7, the results after 2 minutes heating. It can be seen that with the 2 minute heating period, the linearity of the curves and the reproducibility of the duplicate values are much better than with the 5 minute heating. It can also be seen that in each of the six graphs, the intensity of coloration formed by glucose is greater than that formed by dextran. This difference in intensity appeared so constant that it was of interest to calculate the percentage of dextran to glucose absorbance values for each of the six graphs. These calculations are shown in Table 1.

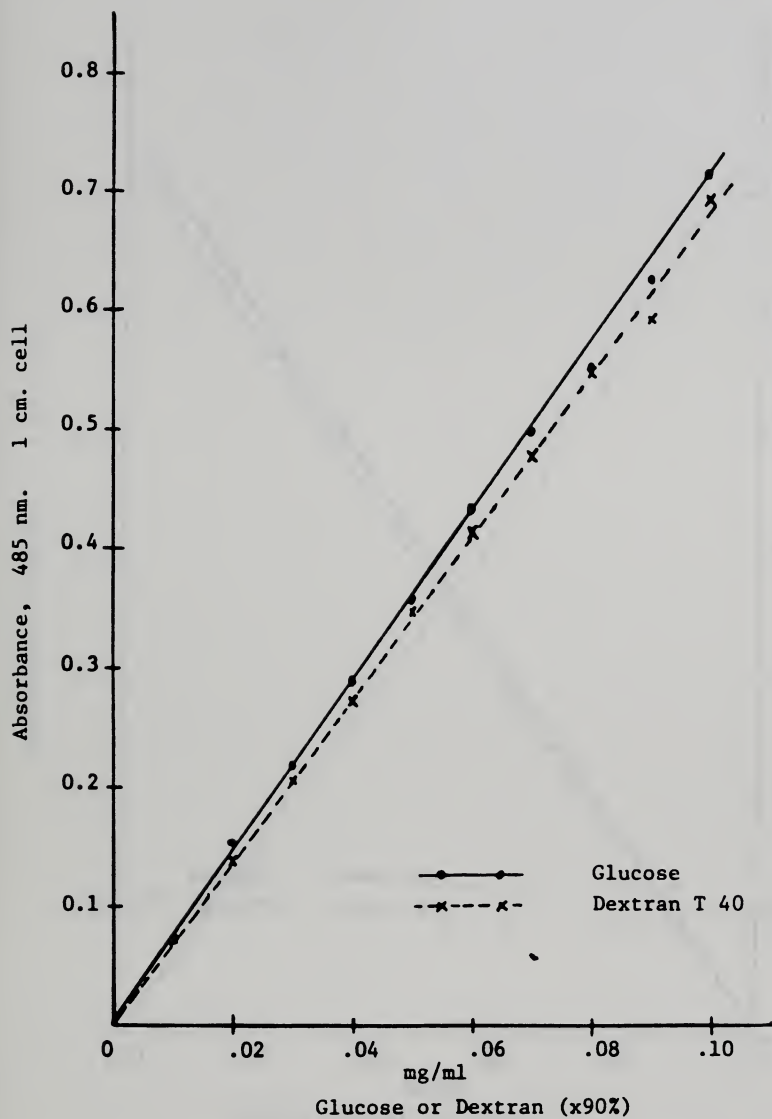


Figure 2.--Percent Dextran T 40 to glucose, 5 min heating.

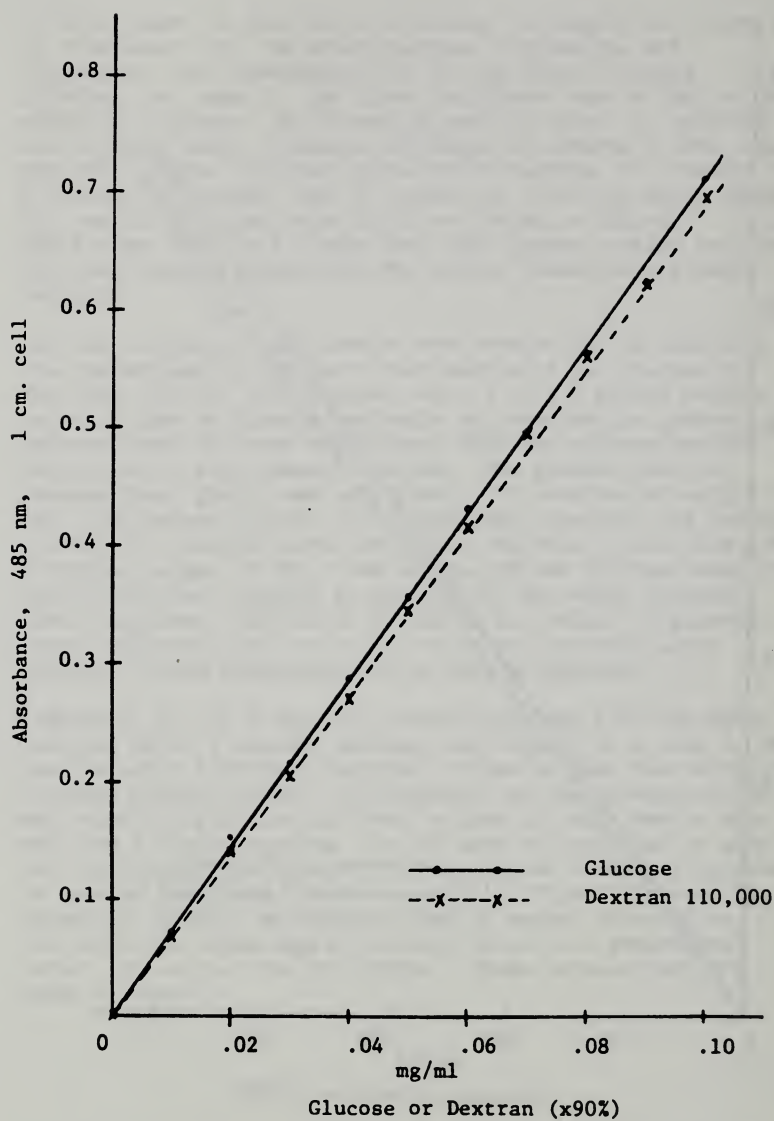


Figure 3.--Percent Dextran 110,000 to glucose, 5 min heating.

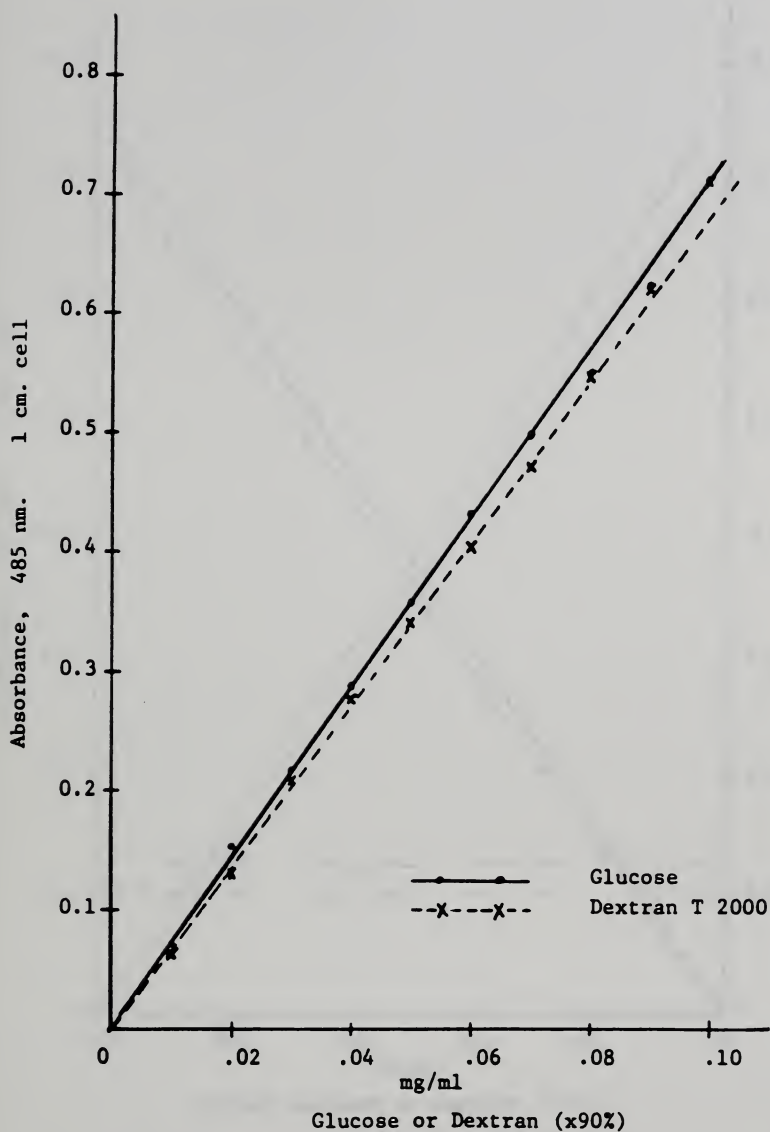


Figure 4.--Percent Dextran T 2000 to glucose, 5 min heating.

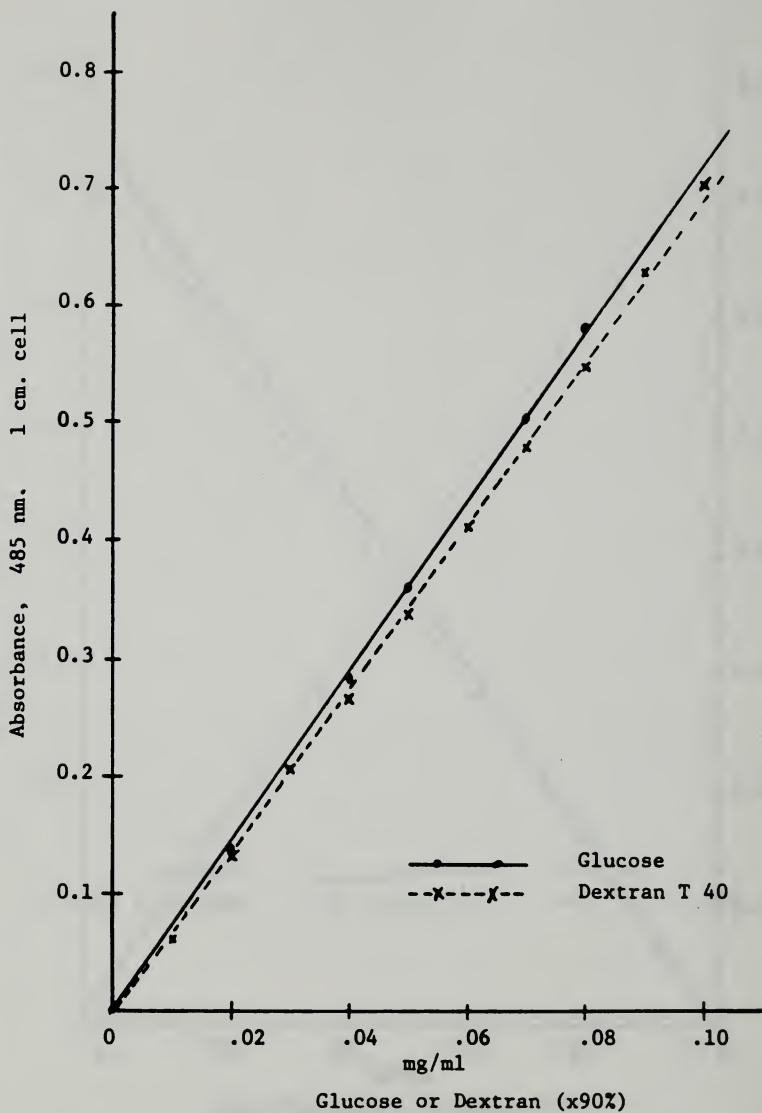


Figure 5.--Percent Dextran T 40 to glucose, 2 min heating.

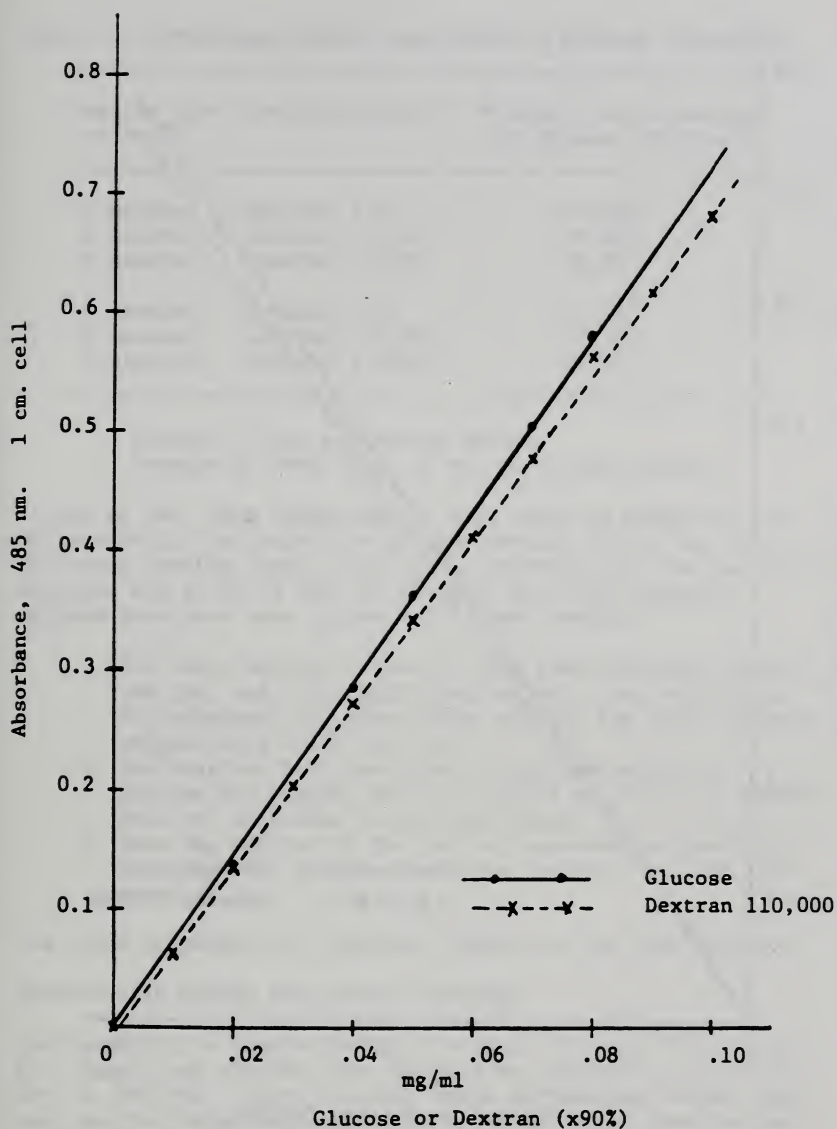


Figure 6.--Percent Dextran 110,000 to glucose, 2 min heating.

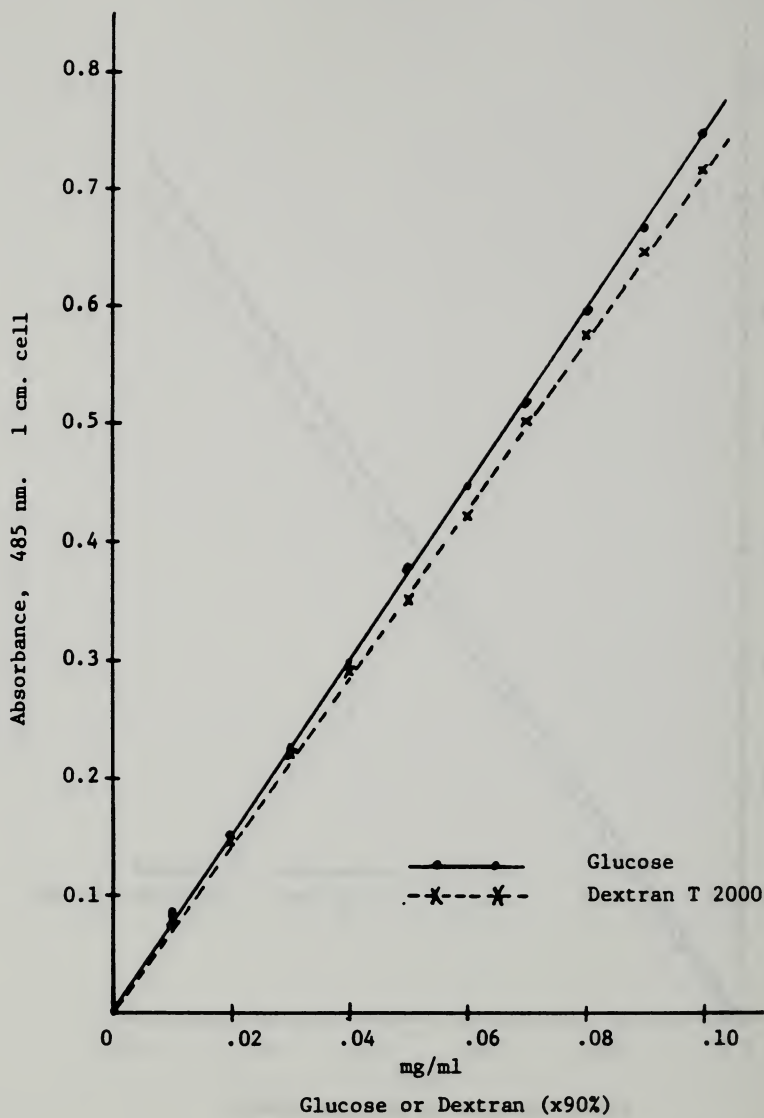


figure 7.--Percent Dextran T 2000 to glucose, 2 min heating.

Table 1.--Percentage dextran absorbance to glucose absorbance

Heating time at 100°C	Polysaccharide	Average % value-dextran to glucose absorbance
2 minutes	Dextran T 40	94.7% ²
2 minutes	Dextran 110,000	95.4% ²
2 minutes	Dextran T 2000	95.8% ¹
5 minutes	Dextran T 40	95.9% ¹
5 minutes	Dextran 110,000	97.2% ²
5 minutes	Dextran T 2000	95.2% ¹

¹ Average of ten percentage values.

² Average of from three to six percentage values.

It can be seen from these results that there is effectively no difference in the dextran to glucose percent values for the different heating times. The colour intensity for the dextrans averaged out at 95.7% that of glucose, and three possible explanations have been proposed for these results.

1. The water content values for the three dextrans were too low, and when additional material was weighed out to compensate for these water values, the final dextran weights were still too low.
2. The dextrans each contained a high percentage of glucose end groups, and the weights of these end groups were not increased during hydrolysis.
3. Some degradation of the dextran occurred on hydrolysis, therefore the weight of glucose produced was less than expected.

The three proposals are discussed further in the next section.

QUANTITATIVE METHOD FOR DEXTRAN ANALYSIS

The quantitative dextran analysis method, of great interest to B.C. Sugar, has already been outlined and described earlier by Earl J. Roberts. There are some basic differences between this test and the total polysaccharide test, one being that Dextran T 2000 is used for standard graph preparation in the former, whereas glucose is used in the latter. As well, the dextran test involves reaction of the dextran T 2000 and the test solutions with a copper containing reagent; the resulting copper-dextran complexes are easily separated from sucrose and invert in the test solutions. In both tests, however, the colour forming reagents are phenol and sulfuric acid.

In our preliminary investigations of the dextran test, we again concentrated our efforts on the evaluation of the phenol-sulfuric acid colour forming step on sucrose-free solutions of polysaccharides. We prepared glucose standard curves (as before), and used weights of dextrans (ie. after adjustments upwards to account for the water contents) which were 90% of the weight of glucose. The copper complexing and detection of the dextran was carried out as described in the dextran test, preparation of standard curve section.

Our first experiment was concerned with the reaction of Dextran T 40, Dextran 110,000, and Dextran T 2000 with the copper reagents, followed by treatment with phenol-sulfuric acid, and a 5 minute 100°C heating period. The colour values were determined and plotted. Figure 8 shows that the three dextran curves are superimposable on each other, and on the glucose standard graph.

Similarly, the reaction of Dextran T 40 and Dextran T 2000 with the copper, phenol and acid reagents was carried out, followed by a 2 minute 100°C heating period. The colour values were determined and again plotted. Figure 9 shows that, as before, the two dextran curves are superimposable on each other, and also on the glucose standard graph.

The fact that the dextran curves are superimposable on the glucose graph only if the copper-dextran complexing step has been carried out suggests that the dextran water content values were accurate, and the percentage of glucose end groups was unimportant (See proposals 1 and 2). It also suggests that the presence of copper has some stabilizing effect on dextrans as they are being hydrolyzed, as little or no degradation occurs (see proposal 3).

CONCLUSIONS

1. The phenol-sulfuric acid colour forming reagents are very effective at hydrolyzing polysaccharides, and detecting the acid breakdown products of sugars.
2. The copper complexing step in the dextran step occurs to the same degree with the three different dextrans.
3. The 2 minute heating period, after the addition of sulfuric acid results in excellent duplicate values, and a linear graph with all points on the graph. The advantages of using this additional heating step apply to both the total polysaccharide and the dextran test.
4. As the percent dextran to glucose value is almost a constant for the three dextrans, a weight of dextran (preferably Dextran T 2000) can be calculated and used for the preparation of the dextran standard graph, without the use of the copper complexing steps, ie. the dextran is detected by reaction with the phenol-acid reagents.

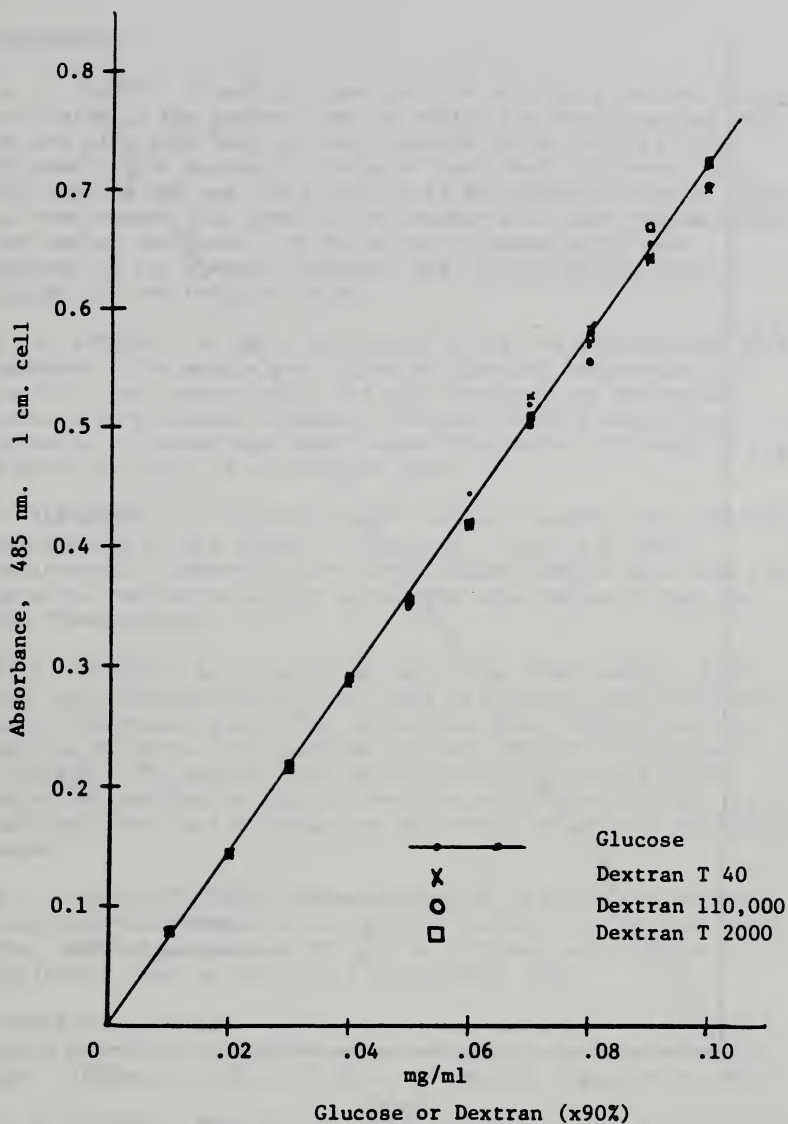


Figure 8.--Dextrans T 40, 110,000, T 2000 and glucose standard curves, 5 min heating.

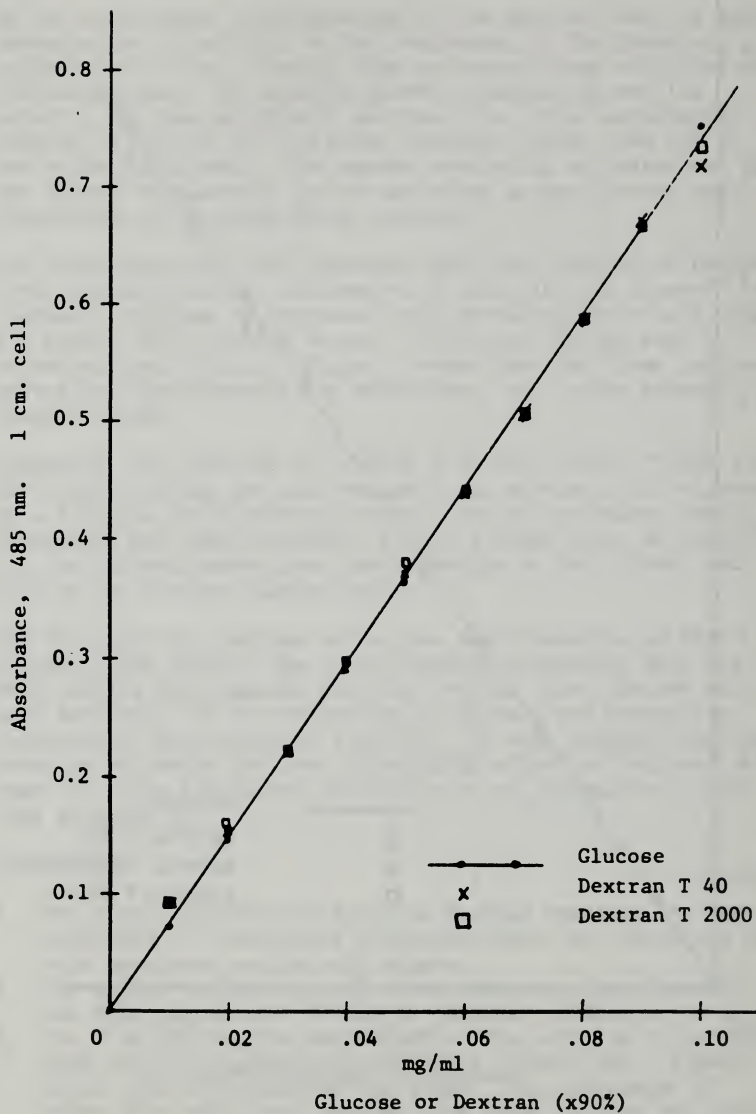


Figure 9.--Dextrans T 40, T 2000 and glucose standard curves, 2 min heating.

DISCUSSION

M. A. CLARKE: I want to thank you for solving a problem of precision in the dextran test by adding the final heating step. We are also glad that you have speeded up the test by using glucose as the standard. We have found that you cannot just dry dextran and use dried dextran as the standard because there is some danger that some of the dextran will have retrograded and become insoluble. It is better to measure the water content of the standard dextran, and, as you pointed out, adjust for the residual water.

R. A. KITCHEN: We never attempted to dry the dextran used as a standard. The sample was stored at constant temperature and humidity, and periodically the water content was determined using a Karl Fischer automatic titrator, methyl alcohol as solvent. A percentage water versus time curve for methyl alcohol was used as a reference graph.

J. ALEXANDER: I congratulate you on the linearity and complete overlapping of the graphs in Figure 8. This is a great achievement. However, your starch values seem to be a bit erratic. Would you expect to get the same values if you used the Plews method?

R. A. KITCHEN: No. I would say not. The Plews method, like the total polysaccharide test, uses an alcohol precipitation and a filtration step. The extraction step, however, is with calcium chloride, and starches are very soluble in calcium chloride. The problem with detecting these polymers by the total polysaccharide test is that the extraction is with boiling water, and starches are not overly soluble in boiling water.

E. J. ROBERTS: I would like to thank Dr. Kitchen for adding some good innovations to our dextran method. I hope that is what everybody that uses it will do. Please send in your refinements and we will get a very useful test.

ANDREW HO (Redpath): Referring to the two graphs in Figures 8 and 9 you mentioned that you used a boiling period of 2 and 5 min. It looks to me as if those two graphs are superimposable.

R. A. KITCHEN: They are not superimposable. The 2 min curve is very slightly higher than the 5 min curve. This is the same effect that was noted with the total polysaccharide test (see Figure 1), except that here the difference between the two graphs is much smaller. What this means is that by first complexing the dextran with copper, the constituents responsible for color formation are being stabilized.

Symposium.--New methods of analysis and sugar processing
technology

ANALYSIS OF SUGAR LIQUORS FOR POLYSACCHARIDES

G. W. Vane

Tate & Lyle, Ltd.

INTRODUCTION

There has been an increasing recognition in recent years of the importance of polysaccharide impurities to sugar manufacturers and refiners. Recent presentations at this and other sugar processing meetings have documented problems caused by such impurities to refiners and consumers of sugar, and these have generally been ascribed to the presence of excessive levels of dextran. Examination of these cases highlighted a major problem: in the majority of cases no conclusive proof was presented that the culprit was actually dextran - rather that the problems were due to "gummy materials", "haze formers", "excessive viscosity" and other such broad terms. This is very simply explained - only very recently have analytical methods become available which are specific to dextran, as distinct from the number of high molecular weight species commonly present in sugar liquors. At this meeting, and the 1981 S I T meeting, we have heard detailed descriptions of two methods of analysis that are specific to dextran; in one case to linear α -1,6 poly glucans only. We have also heard more of the deleterious effects of dextran in sugar processing.

Given the volume of previous work on the effects of dextranase, the problems caused (at least initially) by some mechanical harvesters and recent work using specific dextran analyses and characterised dextrans, it is obvious that the blame ascribed to dextran is largely justified. But it is possible, and I think probable, that other polysaccharide species are involved in some of the problems ascribed to dextran. These may be chemically very similar to dextran, or unrelated (e.g. indigenous sugar cane polysaccharide, ISP). But charged polysaccharides would, for example, be expected to have a proportionately greater effect upon viscosity than a neutral species of similar molecular weight. So it is possible that whilst we may find that a large proportion of the high molecular weight material is dextran, it may not contribute all of the processing problems. It is also possible that

synergistic effects play a part in this; between different polysaccharides or between polysaccharides and other materials.

So it is perhaps timely that we now take a broader view of polysaccharide analysis methods. I have deliberately omitted pure research methods, and looked at methods that are, at least in principle, of regular use in sugar processing laboratories. Thus, no mention is made of techniques such as NMR which can give useful structural as well as chemical information, at the price of time and expensive skills and equipment.

Analysis of Sugar Liquors for Polysaccharides

We must first consider what we are trying to find out from our analytical methods. A prime parameter is the molecular size, generally as molecular weight. We are also interested in the chemical structure, at least from the point of view of ionic charge.

To start with the one I suppose that we all know best - alcohol precipitation. The use of ethanol as a means of precipitating high molecular weight species from sugar liquors. Over the years, it has been developed to the point where, carefully used it can give results which meaningfully reflect the high molecular weight content (less, generally, the starch and protein) of sugar liquors. In my S I T paper in 1981, I presented data which demonstrated that haze analysis on high grade liquid sucrose correlated quite well with the dextran level determined by an immuno-assay method. In less pure sugars, the results will reflect the level of high molecular weight material, which may well be predominantly dextran in many cases.

The method could be further exploited - for it has many advantages in practical terms - if used in conjunction with pure dextranase. Measurements of the haze before and after such treatment ought to afford a specific measure of dextran; there is perhaps a calibration problem, but standards are available, and the method could provide a reliable comparative means which could be used in the process laboratory, with generally available equipment. We have done a little work on this in the past, and did see considerable diminution of the haze following dextranase treatment. I believe a number of other people have reported similar results. Given the increasing availability of relevant enzymes, the method could perhaps be broadened to other polysaccharides.

The specific methods, using anti body precipitation, and alkaline copper sulphate, have been presented in detail here and at the 1981 S I T, and I will not duplicate these presentations at all. It should

be noted that the immuno method is in principle applicable to other polysaccharides, and that the methodology is not complicated once the anti body is available. Rather, a careful clean approach is required, but it should technically be applicable in a process laboratory provided that the polysaccharide/antibody reaction can be easily and reliably monitored.

The remaining area for examining "whole" polysaccharides is chromatographic separation, where information can be obtained on concentration, molecular weight and ionic charge. Previously, we have been limited to gel permeation chromatography (GPC) for example on Sephadex. Whilst the technique requires experience and careful working, it is capable of providing useful information on molecular weight in particular. Using a sensitive detection system such as phenol/sulphuric acid, it can provide molecular weight distributions covering low absolute concentrations.

In the last few years, the scope of such chromatographic methods has been increased by the introduction of gel materials capable of withstanding the demanding conditions of high pressure liquid chromatography (HPLC). These systems allow molecular weight distributions to be obtained in an hour, which on soft gels would have taken a day or more. A number of such column systems are currently available, and we would reasonably expect their range and capability to increase in the future. A key area for further development in these systems is the detection systems. Polysaccharides are normally present in sugar liquors in small absolute amounts, and with a refractive index detector may not give a significant signal - especially if the molecular weight distribution is broad.

For routine use of such HPLC systems by sugar processors, we will in all probability require some more sensitive detection system. The other commercially available HPLC detector (UV absorption) is not very useful for polysaccharide detection in general, though we found this very useful for colourant analysis using one of the high molecular weight HPLC columns.

The columns are capable of separating on a molecular size basis, independantly of charge density, and will provide some measure of molecular weight. Measurements at a range of ionic strengths will yield information on charge properties. Different columns are available for carbohydrates and for proteins, and we have done some work with several of the former type. We happen to have used columns made by the Toyo Soda Company of Japan, but other manufacturers offer column systems of apparently comparable capabilities.

To illustrate the utility of these new columns, I will show some of the results that we have obtained using TSK PW type columns.

We currently have a G2000PW column which resolves mono- and disaccharides and oligo saccharides up to around 10,000 molecular weight. Other columns are available for measurements of molecular weights up to thirty million (based on dextran standards) in broad ranges.

Samples of 3rd crop remelt masses were examined on the G2000 column, using a refractive index detector. We obtained typically the distribution shown in Figure 1, where it is seen that sucrose and invert are resolved, and that a few per cent of the total solids are present with molecular weight $< 10,000$. For these measurements, we simply injected a Millipore filtered solution of the mass at around 10% solids. Over several dozen runs, no change in column performance was noted and the colour on seemed to elute completely. So, given suitable HPLC equipment, it is hoped that this type of column affords a rapid method of at least estimating the high molecular weight material. The detection system is not of course specific to polysaccharides and this must be borne in mind in interpreting the results. Once again, the use of enzymes in conjunction with such columns would seem to offer the basis of rapid, specific polysaccharide analysis.

We have also run some materials on a column system capable of resolving molecular weights up to around seven million. This system uses G5000 PW and G3000 PW columns in series, allowing resolution down to molecular weights of around a thousand.

A sample of ISP supplied by SPRI was examined, in aqueous solution. This showed a mainly bimodal distribution (Figure 2), with a maximum molecular weight higher than seven million. The majority of the material, however, was resolved and showed main peaks at around 300,000 and 10,000, with other material over the whole molecular weight range. It would also be of interest to examine the effect of ionic strength on the molecular weight spectrum.

We also studied a sample of remelt mother liquor from a cane sugar refinery. We ultrafiltered a large batch (50L) of this syrup and washed the concentrate with large volumes of water during ultrafiltration, until the sugar content of the concentrate was $< 2\%$. We then centrifuged the concentrate and decolourised the supernatant with powdered carbon, and finally isolated the soluble solids by freeze drying.

We found the material to be excluded from Sephadex G100 (i.e. $MW > 100,000$). We then ran this material, again as an aqueous solution, on the G5000/3000 column system. This immediately

highlighted the detection problem, for there was a very low response (Figure 2) due to the small quantity of solids applied to the columns. Whilst there is some material around 500,000, there were weak signals in the 6 - 7 million range. At this point we do not know if these were real. We can contrast these results with the Sephadex results, where phenol/ sulphuric acid detection was used on fractions off the column. A clear peak was obtained at V_0 , using the same weight of material as for the HPLC experiments.

So it appears that to fully realise the potential of these new HPLC columns, we need to improve the limits of detection, and also if possible to use detection methods specific to polysaccharides. Perhaps less specific detection, combined with the use of purified enzymes, may provide a rapid practical means of determining the concentration and nature of polysaccharide materials in sugar liquors.

Conclusions

HPLC techniques capable of resolving molecular weights of millions, and other techniques such as hydrolysis/TLC for component sugars, can give much useful information on the nature of polysaccharide impurities. Specific methods are available for the determination of dextran, and the well developed "haze" method has a valuable role as a more general method of estimating the levels of dextran and other polysaccharides. The use of purified enzymes, to selectively degrade different polysaccharide structures, with these techniques offers a rapid means of identifying the chemical nature of these troublesome impurities.

FIGURE 1 - REMELT MASSE / G2000 PW

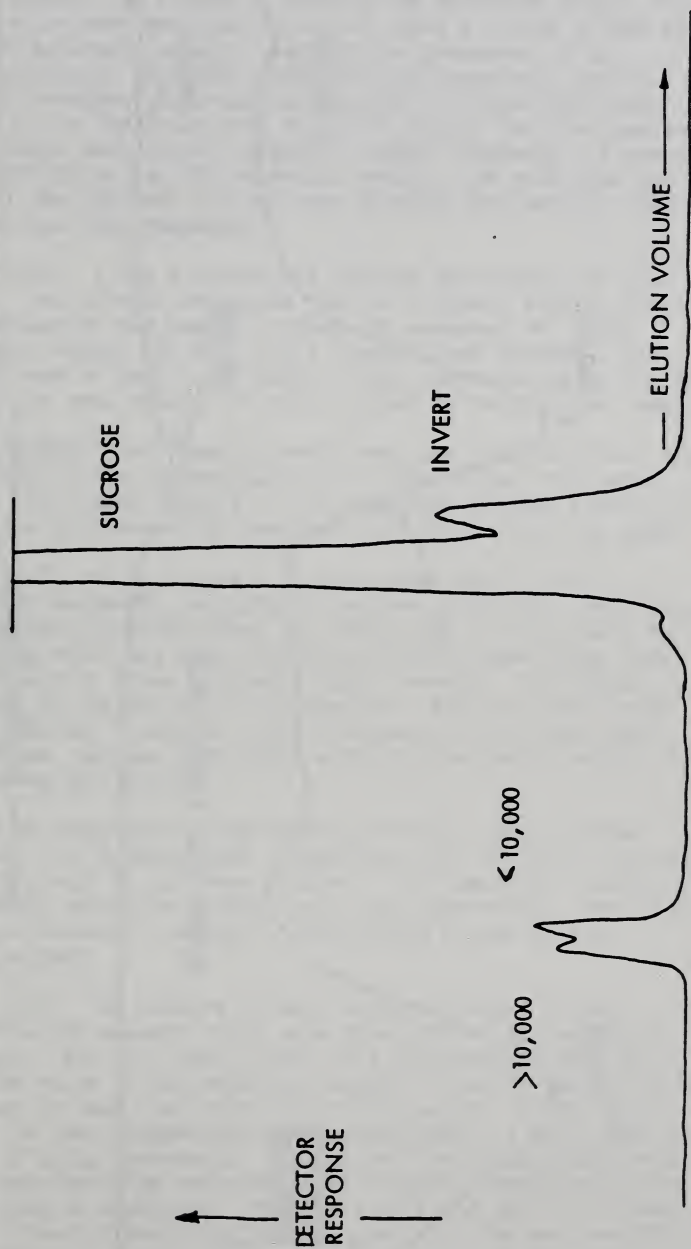
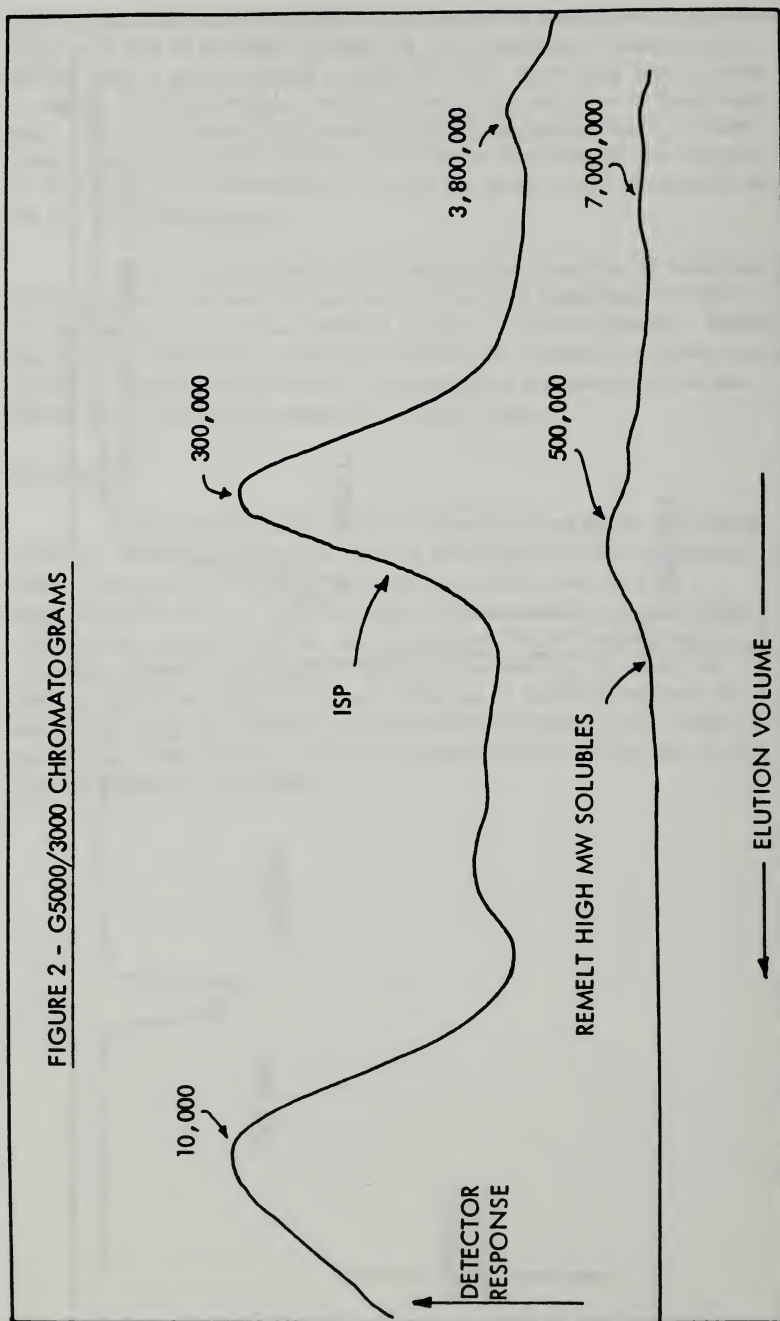


FIGURE 2 - G5000/3000 CHROMATOGRAMS



DISCUSSION

M. A. CLARKE: In Figure 1, showing the molecular weight of the ISP that we sent you, you found two peaks at about 10,000 and 300,000 molecular weight. Part of the treatment in the purification of this ISP, obtained from sugarcane, was an amylase treatment to break down starch. I think that some place in the ISP there must be a 1-4 linkage that the amylase hit forming some 10,000 molecular weight fragments. I expect that 300,000 is the ISP molecular weight. We will send you some of the ISP that has not been treated with amylase, and you can see how that compares.

G. W. VANE: I was a little bit puzzled as to why that peak was there. Mr. Roberts mentioned that in dialysis some of the polysaccharide had passed through the membrane of 12,000 molecular weight cut off, and I thought that perhaps I was seeing some of that. In light of what you were saying the two may be the same thing. We will be glad to have another sample.

E. J. ROBERTS: We found several years ago that invertase does something to the polysaccharide. It hydrolyzes some sugars off of it. This adds support to the idea that amylase could also break the polysaccharide someplace and lead to the two peaks.

G. W. VANE: We have done some work using half a dozen different enzymes. including invertase, pectinase, dextranase, alphaamylase, hemicellulase and cellulase. All of these did something which may mean that there must be some thing like some of all of these polysaccharides present. But the big question is the purity of the enzymes. Before I talk about those results, I want to try the enzymes on different pure substrates, and see what other activity they have besides what it says on the bottle.

ANDREW HO (Redpath): I am working with gel permeation on dextran. The problem that I experience is that it is sometimes very difficult to get pure dextran. The commercial dextran has an average molecular weight, but a big dispersion. How do you assign a molecular weight in identifying a peak in your chromatogram?

G. W. VANE: The numbers I was quoting are based on the manufacturers numbers which were generated using commercial dextrans. You are right, there is a big question over the absolute value of the molecular weight. I can't improve on the information that they offer up to this point. I only have access to those commercial dextrans as well. I don't like to think of going through all the work of trying to fractionate those and determine their molecular weight, perhaps by light scattering or something. That is a very big job - if I were to start it now, I might not have it ready for the next meeting in two years.

Symposium.--New methods of analysis and sugar processing
technology

**ANALYTICAL TECHNIQUES FOR THE SEPARATION OF COLOUR COMPONENTS
AND THEIR APPLICATION IN SUGAR PROCESSING IN CSR FACTORIES**

Peter Smith

CSR Limited

INTRODUCTION

The sugar industry has long recognised that colour removal is the most costly part of milling and sugar refining. CSR has mounted a strong research effort to understand more about the origin and nature of colour in cane sugar products and the effect of processing on this class of impurities.

Cane sugar colour is a complex mixture of diverse chemical components differing in size, charge, ionisation and polar properties. Analytical techniques based on these properties have been developed to separate colour components. Application of the methods to mill and refinery process streams has given us a better understanding of the different colour removal processes than that obtainable with conventional colour measurements. On the basis of this information remedial action may be planned to improve the efficiency of the colour removal processes in milling and refining. The identification of some individual colorants has allowed us to trace their source of origin.

SEPARATION OF COLORANTS FROM SUGAR AND ASH

Colour, although the most visible of impurities in cane sugar products is present only in trace amounts in refinery products. Therefore colorants have to be separated and recovered from sucrose and ash in sufficient quantities to study their composition and physical properties.

The most successful technique we have found to date for recovering cane sugar colorants uses a Rohm and Haas adsorbent resin and was described by Linecar et al.(1978).

This method is the starting point for a number of chromatographic techniques for separating colour components.

The technique has been applied to measure the change in the mass of colour across the milling and refining processes. Concentrations of colorants range from up to 40,000 ppm (based on sugar solids) in the final crushing juice of milling trains in raw sugar factories down to 1,000 ppm in raw sugar and as low as 10 ppm in white sugar (third strike).

Linear relationships at reasonable correlation coefficients between colour attenuation (420 nm) at a fixed solution pH and colour mass have been observed in refineries when the samples were analysed immediately or were stored at 4°C prior to analysis. However, during weekend shutdown when refinery syrups darkened significantly there was no significant change in colour mass (Bardwell et al. 1981). Gel filtration studies indicated that during weekend storage (60°C-80°C) there were molecular rearrangements of polymeric and monomeric colorants to forms which had higher molecular extinction coefficients.

FRACTIONATION OF COLORANTS BASED ON SIZE

The molecular size distribution of cane sugar colorants can be measured by gel filtration techniques using Pharmacia cross-linked dextran gels. G-10, G-25 and LH-20 grades have been used. These gels separate the colorant mixture into polymeric and monomeric fractions. The technique has been particularly useful in studying the molecular size distribution of colorants following refinery carbonatation, mill clarification or in the latter process when excess cane tops have been milled.

In carbonatation, polymeric colorants (MW 1000) of affined raw sugar are largely removed while the alkaline stable and soluble monomeric colorants (pH-sensitive flavonoids) pass into the carbonatated liquor (Kennedy and Smith 1976). Other studies at CSR refineries have shown that where removal of polymeric colour has been efficient in carbonatation, char usage has been low. However carbonatated liquors with the same level of monomeric colorants but with a higher concentration of polymeric colour required more char.

In mill clarification, gel filtration studies revealed that there was also a reduction in the concentration of polymeric colorants (MW 2500) (Smith et al. 1981). However, the reduction in the level of macromolecular colorants was not due to precipitation or physical removal but to a breakdown of polymeric to lower molecular weight colour species. This observation was consistent with the fact that there was little change in the colour attenuation (420 nm) values at pH9 before and after clarification. The reduction in the

polymeric colour fraction was reflected by the lower colour attenuation (420 nm) values at pH4 and 7 of the clarified juice compared with the corresponding unclarified sample. More recent mill trials have shown that there was little change in the mass of colorants before and after clarification indicating that there was no nett removal of colour in this process step.

Gel filtration studies have also shown that when excess cane tops were milled, the polymeric colour fraction and total colour of the clarified cane juice increased (Smith and Gregory 1971). This has important implications in raw sugar quality in that polymeric colorants have a greater propensity to be included in the crystal during mill pan boiling (Smith et al. 1981). Consequently, this class of colorant in raw sugar is less efficiently removed in refinery affination. Clearly cane having considerable amounts of tops and shoots is deleterious in the production of raw sugar having good colour removal properties in refining.

PAPER/THIN LAYER CHROMATOGRAPHY (PC/TLC)

We have had considerable success in identifying the major sugar cane flavonoid pigments in joint studies with Reading University, UK using paper and thin layer chromatography together with UV spectral analysis (Williams et al. 1974). The original studies on leaf material were concerned with the chemotaxonomy and evolution of sugar cane. However this work was also germane to sugar processing technology in that we were able to locate the genetic origin of different flavonoid colorants.

Most commercial cane varieties are hybrids of two basic Saccharum species - S. officinarum (sweet sugar cane) and S. spontaneum (wild cane), with the S. officinarum component making up 80 per cent of the hybrid germplasm. Most of the tricin-based colorants originate from the sweet sugar cane parent, while apigenin type colorants are from the wild cane component of the hybrid. The tricin based colorants are more efficiently removed by bone char and granular carbon than resin, while carbon and resin remove the apigenin colorants more effectively than bone char (Paton and Smith 1982). Current cane breeding strategies appear to be inadvertently reinforcing the wild cane characters in new hybrids by the process of genetic drift (Roach et al. 1981). Thus if these trends continue we could expect the concentration of apigenin based colorants to increase in new cane varieties. The latter when milled, would produce raw sugars requiring more bone char in decolorisation.

Phenolic acids have been identified by co-chromatography of individual acids with reference phenolic acids and by their reactions to certain chromogenic sprays. The phenolic acids

were extracted from acid solutions with ether and the ether extract used for chromatography (Paton 1977). The concentration of the phenolic acids, when extracted, can now be measured by high performance liquid chromatography (Curtin and Paton 1980).

These phenolic compounds, although colorless, are precursors to colour. These impurities, when oxidised or complexed with iron form colour. Laboratory and factory studies have shown that the amount of bone char required to decolorise a carbonatated refinery liquor can be related to the level of the phenolic constituents (Smith et al. 1981).

Application of chromatographic techniques has shown that in mill clarification and refinery carbonatation there was a release of phenolic acids, particularly ferulic acid. The latter has been implicated in white sugar discoloration (Clarke 1980). Phenolic acids were believed to have formed from the hydrolysis of phenolic esters. This observation was consistent with the fact the range of phenolic acids in raw sugar was greater than that detected in unclarified cane juice (Paton 1977).

ELECTROPHORETIC SEPARATIONS

This group of electroanalytical techniques measures certain electrochemical properties of colour and associated impurities. These have to be ionised or charged before they are separated electrophoretically. Three types of electrophoretic separations used in colour studies at CSR are described briefly.

The techniques are not suitable for the recovery of colour components because of the presence of electrolytes used in the separation of the colorant mixture.

Zone Electrophoresis

Charged components are separated on a paper strip which is impregnated with a buffer electrolyte and dipping into electrode tanks containing the same buffer. A high voltage (2KV-5KV) is applied across the paper and the charged species are separated according to their net mobility (speed) and sign of electrical charge (+ or -) in the potential gradient. The pH of the electrolyte buffer can be altered to measure changes in the ratio of anionic and cationic colour species. The separated components are detected as in conventional paper chromatography. The principles of zone electrophoresis are illustrated in figure 1. This technique has been used extensively to separate and catalog a number of colorants using borate buffer as an electrolyte (Farber and Carpenter 1971). At CSR, we have used the technique with acid buffers (pH 2.2) to separate colorants which are combined with ash

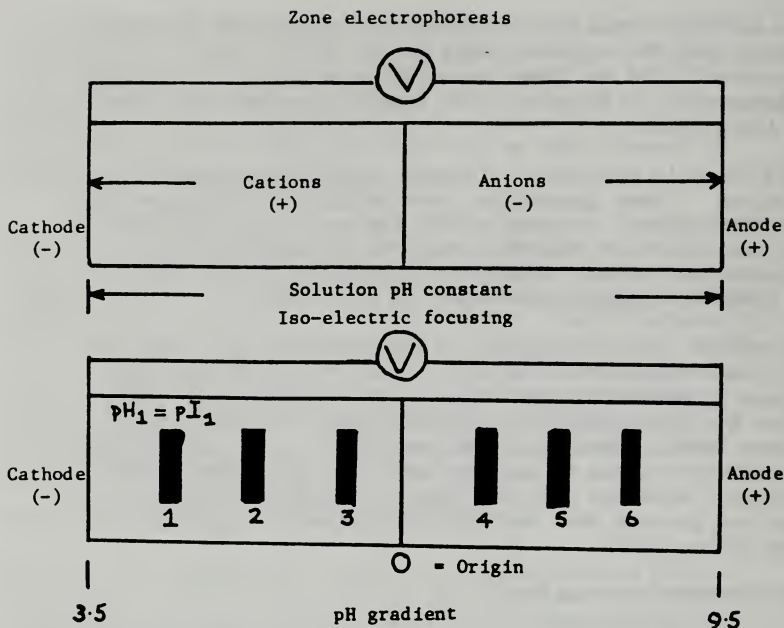


Figure 1.--Principles of zone electrophoresis and iso-electric focusing.

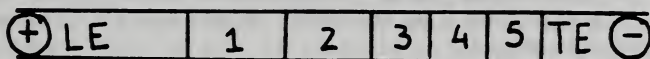
constituents from those that are not. Colorants which are chemically linked to inorganic compounds such as tricin 7-glucosidesulphate migrate when a voltage is applied whereas other colorants which are nonionised, remain stationary under the same experimental conditions.

Iso-electric Focusing

This method can be described as zone electrophoresis in a pH-gradient buffer. In the case of certain colorants and phenolic compounds which are neutral at a given pH (iso-electric point - pI), these will cease to migrate when they enter this pH zone in the gradient buffer for a given applied voltage. Colorant pI values are important parameters in ion exchange decolorisation studies. Below the pI value a colorant is cationic, and above it is anionic. The principles of the technique are illustrated in figure 1 for sugar cane impurities which are zwitter ions.

LKB (Sweden) have designed instruments to separate components by the iso-electric focusing principle. This can be done by column or thin layer chromatographic techniques. Low molecular weight ampholytes, which are basically a mixture of aliphatic polyaminopolycarboxylic acids, are used to produce a stable and linear pH-gradient in the column and thin layer systems. Sugar cane factory colorants have been separated by this technique since they behave identically to commercial ampholytes in iso-electric and isotachopheretic experiments. This is not unexpected as certain factory colorants such as melanoidins are similar in chemical structure. Factory colorants have pI values ranging from 5-6. Thus these impurities are in the cationic form below pH5 and are anionic above pH6. Phenolic colorants are neutral at low pH and become more negatively charged (anionic) with increasing solution pH. Therefore these cannot be separated by iso-electric focusing techniques.

Separation in Tachophor teflon tubing



Isotachopherogram - UV elution profile

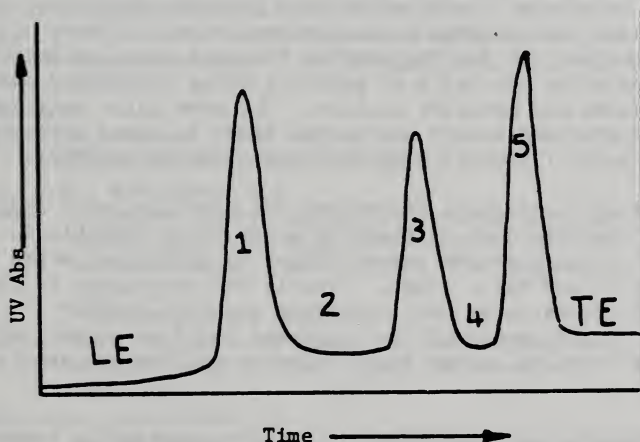


Figure 2.--Idealised isotachopheretic separation of colorants of factory and cane plant origin. 1, 3 and 5 are UV absorbing factory colorants. 2 and 4 are low or non-UV absorbing factory colorants. 2 and 4 act as "spacers" between 1, 3 and 5.

Isotachophoresis

This technique is in essence another form of zone electrophoresis. Here the speed of the ionised components is governed by a faster leading electrolyte (LE) and a slower terminal electrolyte (TE) under a given voltage gradient. The principal effect of isotachophoretic separation is a concentration of all ions with the same speed (net mobility) into separate and distinct zones. These ions then migrate with the same constant velocity as dictated by the leading and terminal electrolytes of the system. LKB (Sweden) have developed an instrument called the Tachophor which operates on this principle. Here the separation takes place in a thermostatically controlled teflon capillary with electrolyte reservoirs and not on a paper chromatogram as in conventional zone electrophoresis. The separated components are detected by UV and thermal monitors. The speed at which the separated components pass the detectors can also be modified. Factory colorants can be readily distinguished from colorants of plant origin by this technique as shown in figure 2. The method is quantitative, with the concentration of the components proportional to the width of the peaks or bands in the isotachopherogram.

High Performance Liquid Chromatography (HPLC)

HPLC allied with solvent extraction and colour solids recovery techniques has been the most powerful analytical tool we have used to date in our laboratory and factory colour studies. In our HPLC method the cane sugar colorants are separated on the basis of polarity using a reversed phase phenyl column and gradient elution. The more polar compounds such as factory colorants are eluted first followed later by the less polar flavonoid pigments (Paton and Smith 1982).

Where solvent extraction procedures are specific for a single class of impurity such as phenolic acids it is possible to separate and measure the concentration of individual acids by HPLC (Curtin and Paton 1980). Clearly the resolution of the flavonoid colorants by this technique could be further improved if this group of cane pigments could be selectively extracted from the colour solids mixture or from the sample itself.

The HPLC technique and its application in identifying types of colorants and their concentration removed from carbonatated refinery liquor by three different decolorisers in CSR refineries, has already been the subject of a paper presented at this meeting. At present we are using the method to monitor changes in the concentration of different flavonoid and phenolic colorants from the milling of the cane plant through to the production of white sugar in refineries. It is hoped that information obtained in this study will help us

to identify and locate the origin of those colorants which are difficult to remove in refining. These may originate in the milling process or in the cane plant.

CONCLUSION

The colour separation techniques used in studies at CSR on factory produced liquors have given us a better understanding of the different colour removal processes in our refineries.

The techniques have enabled us to define colour problems more specifically than would have been possible with conventional colour measurements. If the underlying cause of the colour problem is understood then it may be possible to plan some remedial action to eliminate or reduce it.

Progress in our knowledge of the composition of cane sugar colorants has been dependent on the resolution and sophistication of the techniques applied in our colour studies. For example with the application of HPLC in this area we anticipate that the separation and measurement of the concentration of individual colorants will eventuate in the near future. Thus it will be possible to study the various colour removal steps in milling and refining at these levels.

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Symposium.--New methods of analysis and sugar processing
technology

CONTROL LABORATORY

J. F. Dowling

Refined Sugars

During this conference on sugar processing research, we have dealt mainly with methods relating to dextran, color compounds, etc., and now this symposium is to look at newer methods of analysis being used in the industry. I would like to direct my discussion to the improvement of methods used in the Process control Laboratory area.

The Process Control Lab is faced with the problem of monitoring production with rapid and reproducible methods used by laboratory technicians, who, in most cases, are high school graduates and not PhD's. The methods used must be simple, accurate, and rapid. Thus through the years this lab has looked for methods relying heavily on instrumentation and automation, eliminating, where possible, the lab technician's human error.

In controlling process, the characteristics we are mainly concerned with are Brix, color, pH, ash, invert and sucrose content.

Ash is now being readily estimated by conductivity, applying various conversion factors. Although these factors vary with various origin raw sugars, for general purposes, the method is rapid and accurate enough. The pH can be readily determined with potentiometric pH meters. In this case, the key element is the type of electrodes being used.

Color can be measured by rapid visual methods (Horne Scale, etc.) or by the ICUMSA spectrophotometer methods. Invert determinations by the Lane-Eynon method are fairly rapid and accurate, but newer instruments relating to the use of enzymes or HPLC may soon find a place in the Control Lab.

The two areas that I would like to talk about in more depth are the automation of remelt pots and continuous sugar content measurement.

Back in the 1960's we started using Auto-analyzers from Technicon to monitor condensate return to the powerhouse and sewerage for sugar loss. Presently, we have three main monitoring points for sugar loss. Our main sewer lines are tied into one sewerage pit which is monitored by an original model Auto-analyzer. We have been replacing parts on this system by buying used and rebuilt parts. This old model is preferred because of the larger cell, which allows for greater error in prefiltering the sample. The sensitivity of this unit is 0 to 4 Brix, with hydrochloric acid and resorcinol reagents. Problems in this area are related to filtering the sample and color burn at high brix.

The river water used in our vacuum system which is returned to the river is monitored with a newer Technicon Monitor IV unit. The sensitivity of this unit is 0 to 500 ppm with hydrochloric acid, sodium carbonate and Neocuproine Copper Reagent. Problems in this area have been related to very small cell size, spills within the instrument, and the generally delicate nature of the instrument for plant usage.

Our powerhouse return is now monitored by a Scientific Instruments Corp. Continuous Flow Analyzer. These units are similar in principle to the Technicon instruments but appear to be better suited for plant usage. The unit can be contained in one spill controlled cabinet or separated to measure various lines with one pumping system. The design of the pump is such that longer life is expected. To date, we have had one of these units on line for four months with no major problems. The chemistry presently being used is concentrated sulfuric acid and resorcinol for detection of 0 to 100 ppm. There is no heating bath required in this reaction since the heat generated by the addition of sulfuric acid is sufficient to invert the sucrose present.

In general, I feel that auto-analyzers are an excellent means of continuously monitoring sugar loss; however, the need for plant oriented instruments still exists.

We monitor our remelt recovery operation mainly by apparent purity. In recent years this has been somewhat automated by addition of the automatic polarimeter. We have a Rudolph Automatic Saccharimeter and have been very happy with its performance in eliminating the operator error in balancing the light fields. One of the major possible errors in determining purity is the measurement of Brix. We presently determine all control Brix on a Bausch & Lomb Abbe 3L Refractometer. However, in dealing with dark liquor, it is often hard to obtain a clear shadow. Last year digital direct reading

refractometers became available in the United States. Anacon manufactures such a unit, which we have under test for two weeks. The unit automatically balances the shadow and prints a temperature corrected Brix. To date we have measured 24 samples, ranging in brix from 30 to 76. Each sample was tested at least six times by various lab technicians on each refractometer. The average Brix was determined and the average difference calculated disregarding the sign of the difference. The average difference for all samples (150 samples) for the Anacon was 0.13 and for the Abbe also 0.13. Thus the initial testing would indicate that the two refractometers are similar. We have not yet evaluated the effects of darker colored liquors or high processing temperatures. The Anacon model requires greater care in cleaning of a larger prism, but the savings on the technician's eyes could be well worth it.

I feel the industry should continue to investigate and encourage the development of more automated analyses for process control labs in order to eliminate the human error and, in the long run, to reduce operating costs of the labs.

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Autopol IIS	Rudolph Research Fairfield, NJ

DISCUSSION

E. J. CULP: I would like to offer the comment that the beet sugar industry in the U. S. has been concentrating on this field of automating laboratory work. Have we had any feedback from the beet sugar industry into the cane sugar industry?

J. F. DOWLING: As you know, they do the automation on the beets coming in. I don't know of anybody having a completely automated lab for the cane coming in. Dupont has an automated in line polarimeter running one of their plants, but it is not sugar. We had an automatic polarimeter some years ago on invert. But, nobody in the cane sugar business has a completely automated lab where a computer brings all of the various

instrument readings together and prints out the final result. This is what we all want and what we all strive for.

C. C. CHOU: Have you tried a commercially available automated screen analysis based on a laser beam? We all do a lot of screen analysis, and if we could do something in this area, we could save a lot of manual work.

J. F. DOWLING: No. But have you tried the coulter counter?

C. C. CHOU: We tried it but it takes too much time and you also need a non-aqueous solvent. Incidentally, have you tried HPLC in a production lab for invert analysis?

J. F. DOWLING: I do not have an HPLC. All the corn refiners are monitoring the levulose with HPLC in the control labs. So, HPLC is a control tool and it should be excellent for sucrose, but it needs to be a little more quantitative.

C. C. CHOU: We tried very hard to analyse for invert in refining process streams. We have a complete evaluation of a column from Waters and one from Bio-Rad. The results are not promising.

I want to extend a challenge to this group, particularly to those who have the authority and responsibility to formulate research projects. I would like to see some work done on the kinetics of bone char regeneration. All that a refinery is doing is removing 0.1% to 0.3% of material that is causing color, either by bone char, clarification, crystallization, etc. It is essential to have test methods to monitor the performance of the char regeneration process if we are to succeed in reducing char decolorization cost. There are many instruments available to do the work such as thermal gravimetric analysis, DTA, DSC, etc.

J. F. DOWLING: It is difficult to measure invert at the level found in refineries with such a huge sucrose peak with HPLC. But there is another method; the enzymatic conversion and then the measuring of hydrogen peroxide. I guess that several of us here are aware of this method, but have never tried it.

A. B. RAVNO: Many of the beet sugar labs in Europe use the enzymatic method for glucose analysis. At the Berlin Institute they are working on a redox potential method for invert.

S. E. GEORGE (BC Sugars): We are in the beet business as well as in the cane business. The level of beet technology in Europe is very much ahead of North America. It is just that they have a lot more money. I think that we should try to get some of the European beet companies into this group because they could bring a lot of good information to us.

M. A. CLARKE: There have been results published comparing the enzyme method (Enzymax) with HPLC and GLC.

M. A. GODSHALL: That work was recently published in JAOAC (65:126-131, 1982) and was concerned with strawberries. There have been several other recent comparisons of these three methods, with quite good results. Also there is an instrument available from the Yellow Springs Instrument Co that uses the enzymatic method. I know of at least one beet company uses it, and they are very satisfied.

M. A. CLARKE: In the use of HPLC for very low levels of invert in the large amount of sucrose, there is another approach involving post column derivatization. A reaction is performed on the separated components, and the reaction products measured spectrophotometrically. This offers a much better chance of determining very low levels of invert in sucrose than by refractometric analysis.

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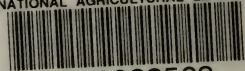
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